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URAD REPUBLIKE SLOVENIJE ZA INTELEKTUALNO LASTNINO

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NOVI DERIVATI PIRIDILETANOL (FENILETIL) AMINOV KOT INHIBITORJEV BIOSINTEZE HOLESTEROLA, POSTOPKI ZA NJIHOVO PRIPRAVO IN FARMACEVTSKI PRIPRAVKI, KI JIH VSEBUJEJO

Ljubljana, 23.4.2003

Janez Kukec-Mezek
svetovalec Vlade

REPUBLIKA SLOVENIJA MINISTRSTVO ZA GOSPODARSTVO
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LJUBLJANA
13

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URAD REPUBLIKE SLOVENIJE
ZA INTELEKTUALNO LASTNINO
LJUBLJANA, KOTNIKOVA 6

ZAHTEVA ZA PODELITEV PATENTA

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Potrdilo o prejemu prijave
(izpolni urad)

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Številka prijave: P-200200177

Žig urada in podpis:

2. Prijavitelj (priimek, ime in naslov, za pravne osebe firma in sedež):
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6. Podatki o zahtevani prednostni pravici in podlagi zanjo:

7. Dodatne zahteve:
- ☐ prijava je za patent s skrajšanim trajanjem
- ☐ predhodna objava patenta po preteku _____ mesecev
- ☐ prijava je izločena iz prijave številka: __________

8. Izjava:
- ☐ izjava o skupnem predstavniku:

9. Priloge:
- ☒ opis izuma, ki ima _18_ strani 2x
- ☒ patentni zahtevek (zahtevki), ki ima(jo) __3__ strani; število zahtevkov: _11___ 2x
- ☒ skice (če so zaradi opisa izuma potrebne); število listov: _9___ 2x
- ☒ povzetek
- ☒ potrdilo o plačilu prijavne pristojbine - sledi
- ☐ potrdilo o deponiranju biološkega materiala, če gre za izum, ki ga ni mogoče drugače opisati
- ☐ pooblastilo zastopniku
- ☐ generalno pooblastilo zastopniku je deponirano pri uradu pod št.: ________
- ☐ potrdilo o razstavni prednostni pravici
- ☐ podatki o drugih prijaviteljih
- ☐ podatki o drugih izumiteljih
- ☐ prikaz zaporedja nukleotidov ali aminokislin v opisu
- ☐ prijava je bila predhodno posredovana po faksu ali v elektronski obliki
- ☐ ________

REPUBLIKA SLOVENIJA
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URAD RS ZA INTELEKTUALNO LASTNINO

Prejeto dne: 17 -07- 2002	Osebna oddaja: ☐
Podpis:	Oddano priporočeno dne: 16 -07- 2002
Šifra:	Poštna številka:

LEK d.d.
KOŠAK ALENKA

Priimek in ime ter podpis prijavitelja (zastopnika)

Obrazec SIPO P-1

Novi derivati piridiletanol (feniletil) aminov kot inhibitorji biosinteze holesterola, postopki za njihovo pripravo in farmacevtski pripravki, ki jih vsebujejo

Področje tehnike v katero spada izum

(MPK C 07 D 213/38, A 61 K 31/44)

Pričujoči izum spada v področje zdravilnih učinkovin iz skupine heterocikličnih spojin in farmacevtske industrije in se nanaša na nove derivate piridiletanol (feniletil) amina, postopke za njihovo pripravo, farmacevtske pripravke, ki jih vsebujejo in na njihovo uporabo za inhibicijo biosinteze holesterola. Novi derivati piridiletanol (feniletil) amina v smislu izuma so ligandi sigma receptorjev, inhibitorjev biosinteze holesterola na stopnji sterolne Δ7,8 – izomeraze in so primerni za zdravljenje hiperholesterolemije in hiperlipemije pri ljudeh.

Tehnični problem

Obstaja stalna potreba po novih učinkovinah kot inhibitorjev biosinteze holesterola, učinkovitih za zdravljenje hiperholesterolemije in hiperlipemije, s katerimi bi dosegli bolj ciljno delovanje v terapiji in z manj stranskimi učinki glede na terapevtske učinkovine, poznanih v stanju tehnike.

Stanje tehnike

Zaradi vpliva visokega nivoja holesterola v krvi na nastanek ateroskleroze je bilo izdelanih že veliko raziskav pri iskanju učinkovin, ki bi zniževala nivo holesterola v krvi sesalcev in bi bile zato učinkovite pri zdravljenju hiperholesterolemije in hipolipemije. Ugotovljeno je bilo, da je eden izmed takšnih načinov zdravljenja z učinkovinami inhibicija biosinteze holesterola.

Poznano je več inhibitorjev biosinteze holesterola na stopnji inhibicije 3-hidroksi-3-metil-glutaril koencim A reduktaze (HMG-CoA reduktaze), kot opisano na primer v US patentu št. 4,231,938 (lovastatin), US patentu št. 4,444,784 (simvastatin) US patentu št. 4,346,227 (pravastatin natrij) ali US patentu št. 5,273,995 (atorvastatin), ki se že uporabljajo v terapiji in so poznani kot komercialni preparati Mevacor®, Sinvacor®, Lipitor®. Navedeni inhibitorji HMG-CoA reduktaze, ki so poznani tudi s skupnim imenom statini, zelo znižajo vsebnost holesterola v krvi.

Iz US patenta št. 4,800,206 so poznani derivati piridin – etanolamina, ki so primerni za zdravljenje debelosti in / ali diabetesa, zlasti pri tolstih odraslih osebah.

Znano je, da se sigma ligandi vežejo na sigma receptorje, ki so strukturni homologi sterolne Δ8,7-izomeraze (F.F.Moebius in drugi, Brit.J.Pharmacol. (1997), 121, 1-6), ki nastopi v zadnjih stopnjah biosinteze holesterola, vendar v medicini še niso znane učinkovine in zdravila, ki bi inhibirala biosintezo holesterola na tej stopnji.

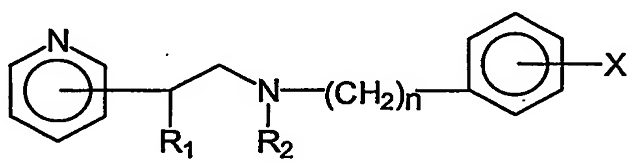
Opis rešitve tehničnega problema z izvedbenimi primeri

Izum temelji na nalogi najti nove učinkovine, ki bi signifikantno znižale vsebnost holesterola v krvi sesalcev na osnovi inhibicije biosinteze holesterola na zadnjih stopnjah njegove biosintetske poti in sicer na stopnji sterolne Δ7,8 - izomeraze, kar bi bilo bolj selektivno od vpliva znanih statinov, ki inhibirajo HMG-CoA reduktazo, ki nastopi v zgodnji fazi biosintetske poti holesterola.

Z novimi spojinami v smislu izuma bi dosegli bolj ciljno delovanje in z manj stranskimi učinki kot jih imajo v terapiji že uveljavljene učinkovine.

Ta problem smo rešili s pričujočim izumom, ki se nanaša na nove derivate piridiletanol (feniletil) amina, postopke za njihovo pripravo, na farmacevtske pripravke, ki jih vsebujejo in uporabo novih spojin v smislu izuma za zdravljenje hiperholestrerolemije in hipolipemije.

Novi piridiletanol (feniletil) amini v smislu izuma so spojine s splošno formulo I



I

kjer pomeni

n je celo število od 1 do 4
R_1 je vodikov atom, hidroksilna skupina ali nižja C_{1-6} alkoksi skupina
R_2 je vodikov atom ali nižja C_{1-6} alkilna skupina z ravno ali razvejeno verigo
X je vodik, fluor, klor, brom, hidroksilna skupina, trifluorometilna skupina, 3,4-di-Cl , 2,4-di-Cl ali nižja C_{1-6} alkoksi skupina

kot tudi njihove fiziološko sprejemljive kislinske adicijske soli.

Izraz nižja alkilna skupina pomeni nižjo alkilno skupino z ravno ali razvejeno verigo z 1 do 6, prednostno z 1 do 4 ogljikovimi atomi (C_{1-6} alkil), kot so metilna, etilna, n-propilna, izopropilna, n-butilna in izobutilna skupina. Izraz nižja alkoksi skupina pomeni alkoksi skupino z 1 do 6, prednostno z 1 do 4 ogljikovimi atomi (C_{1-6} alkoksi), kot so metoksi, etoksi, propoksi, izopropoksi, butoksi in izobutoksi skupina.

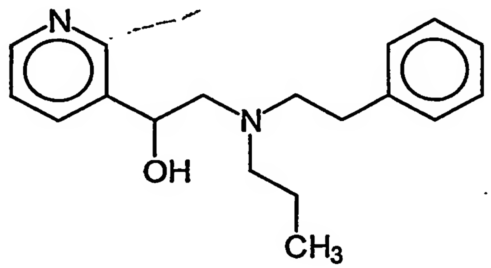
Spojine s formulo I tvorijo soli s kislinami in te soli so tudi del izuma. Primeri takšnih soli so soli s fiziološko sprejemljivimi mineralnimi kislinami kot so na primer klorovodikova kislina, bromovodikova kislina, fosforjeva kislina; ali z organskimi kislinami kot so na primer metansulfonska kislina , citronska kislina, oksalna kislina, maleinska kislina, benzensulfonska kislina in druge.

Nove spojine v skladu s smotrom izuma imajo vsaj en asimetrični ogljikov atom in lahko zato obstojajo kot optično aktivne enantiomere, kot diastereomere ali kot racemati.

Spojine s formulo I, kjer pomeni n = 2 in v katerih je R_1 hidroksilna skupina, R_2 je metilna ali n-propilna skupina in pomeni X vodikov atom ali dva atoma klora na mestih 3 in 4 fenilnega jedra, so novi derivati piridiletanol (feniletil) amina in so prednostne spojine v smislu izuma.

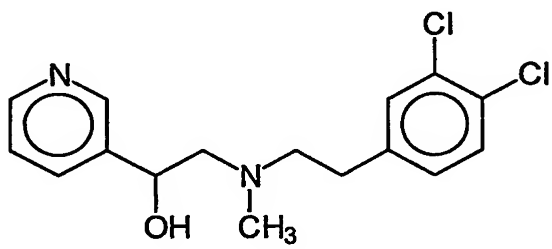
Izmed gornjih spojin so prednostne sledeče spojine:

1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino) etanol in njegova dihidrobromidna sol s formulo II (označena v nadaljnem opisu in slikah kot BK-31)



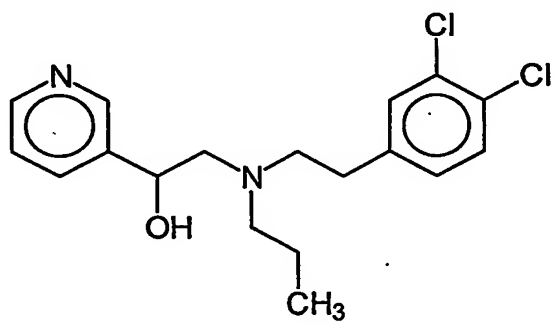
II

1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanol in njegova dihidrobromidna sol s formulo III (označena v nadaljnjem opisu in slikah kot BK-33)



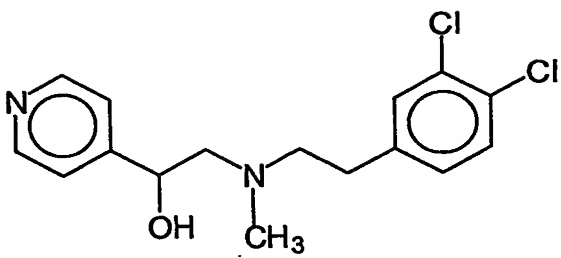
III

1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol in njegova dihidrobromidna sol s formulo IV (označena v nadaljnjem opisu in slikah kot BK-35)



IV

in 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanol in njegova dihidrobromidna sol s formulo V (označena v nadaljnem opisu in slikah kot BK-38)



V

Izmed zgoraj navedenih spojin v smislu izuma je zlasti prednostna spojina 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol in njegova dihidrobromidna sol (BK-35.2HBr) kot inhibitor biosinteze holesterola in s tem primerna pri zdravljenju hiperholesterolemije in hiperlipemije.

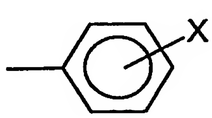
Spojine v smislu izuma lahko pripravimo na dva različna načina, ki sta ponazorjena na sledeči shemi kot varianta (a) in varianta (b) in sicer :

varianta a):

alkiliranje sekundarnih aminov s formulo VI

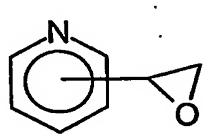
$$NHR_2CH_2CH_2Z \qquad \text{VI}$$

v kateri ima R_2 zgoraj naveden pomen in Z pomeni skupino



v kateri ima X zgoraj navedeni pomen,

s piridil oksiranom (piridin etilenoksid) s formulo VII



VII

do želenih naslovnih piridiletanol (feniletil) aminov s formulo I, ki jih po želji presnovimo v njihove fiziološko sprejemljive kislinske adicijske soli.

Sekundarne amine s formulo VI lahko pripravimo z alkiliranjem primarnih aminov s formulo XII

$$H_2N - CH_2CH_2Z \qquad \text{XII}$$

z alkil jodidi s formulo XIII

R_2J . XIII

po sledeči reakcijski shemi:

$$H_2N-CH_2CH_2Z \quad + \quad R_2J \longrightarrow HNR_2-CH_2CH_2Z$$

kjer imata substituenti R_2 in Z zgoraj naveden pomen.

Primarni amini s formulo XII in alkil jodidi s formulo XIII so znane in komercialno dostopne kemikalije.

Na mestih 2, 3 ali 4 substituirane piridiloksirane s formulo VII v postopku alkiliranja sekundarnih aminov s formulo VI pripravimo *in situ* s presnovo na mestih 2, 3 ali 4 substituiranega bromoacetilpiridin hidrobromida s kompleksnimi kovinskimi hidridi, kot z natrijevim borhidridom v inertnem topilu kot nižjem alifatskem alkanolu, na primer etanolu, pri temperaturi okoli sobne.

Na mestih 2, 3 ali 4 substituiran bromacetilpiridin hidrobromid pripravimo s presnovo izhodnega na mestih 2, 3 ali 4 substituiranega acetilpiridina, ki so znane in komercialno dostopne kemikalije, z bromiranjem z bromom in bromovodikovo kislino.

Postopek alkiliranja sekundarnih aminov s formulo VI s piridiloksirani s formulo VII poteka pri temperaturah od okoli sobne do temperature refluksa reakcijske zmesi, v inertnem topilu kot nižjem alifatskem alkanolu, na primer etanolu. Dobljene surove piridiletanol (feniletil) amine s formulo I izoliramo in očistimo na znane načine, najbolje s kolonsko kromatografijo.

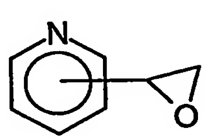
Varianta (b) :

alkiliranje primarnih aminov s formulo VIII

R_2NH_2

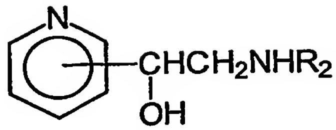
v kateri ima R_2 zgoraj naveden pomen,

s piridil oksiranom s formulo VII



VII

do intermediarnih spojin s formulo IX



IX

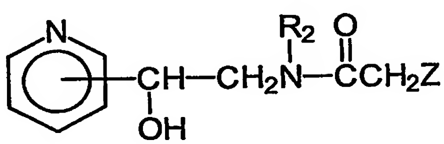
v kateri ima R_2 zgoraj naveden pomen,

ki jih kondenziramo z derivati fenil ocetne kisline s formulo X

$$HOOCCH_2Z \qquad X$$

v kateri ima Z zgoraj naveden pomen,

do novih intermediarnih spojin s formulo XI



XI

v katerih imata substituenti R_2 in Z zgoraj naveden pomen,

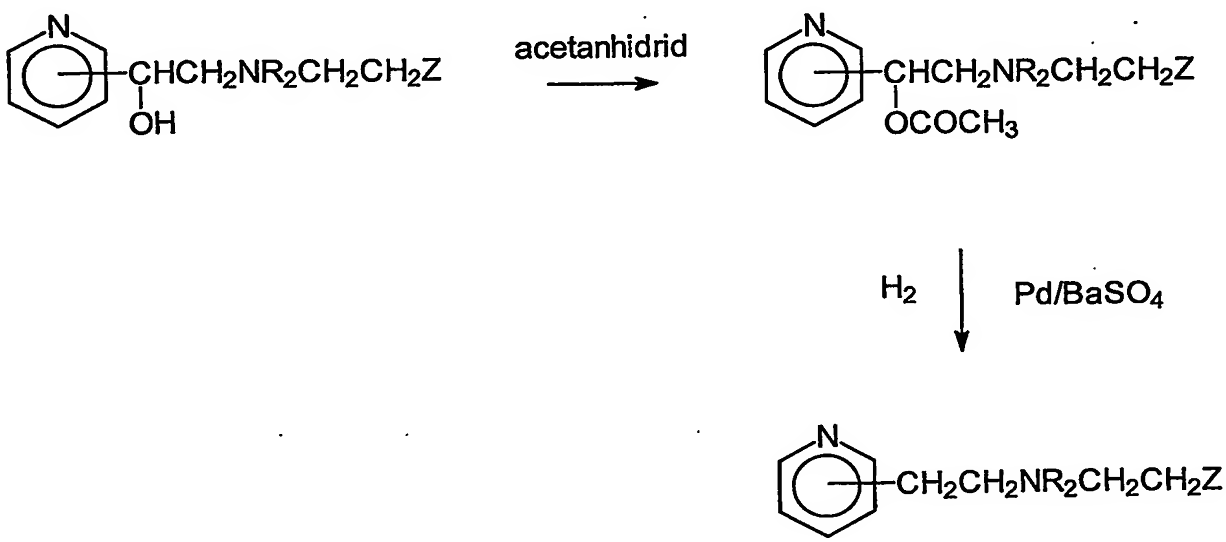
ki jih reduciramo do želenih naslovnih piridiletanol (feniletil) aminov s formulo I, ki jih nato po želji presnovimo v njihove fiziološko sprejemljive kislinske adicijske soli.

Primarni alifatski amini s formulo VIII, kot metilamin ali n-propilamin, so znane in komercialno dostopne kemikalije, ki jih alkiliramo s piridil oksiranom s formulo VII v inertnem topilu, kot nižjem alifatskem alkanolu, na primer etanolu, do intermediarnih spojin s formulo IX. Slednje intermediarne spojine kondenziramo z derivati fenil ocetne kisline s formulo X, kjer ima substutuenta Z zgoraj naveden pomen, v inertnem topilu in pri temperaturi okoli sobne. Kot kondenzacijsko sredstvo lahko uporabimo znana in v literaturi opisana kondenzacijska sredstva, kot diciklohekslkarbodiimid (DCC), kot inertno topilo na primer metilen klorid (diklorometan).

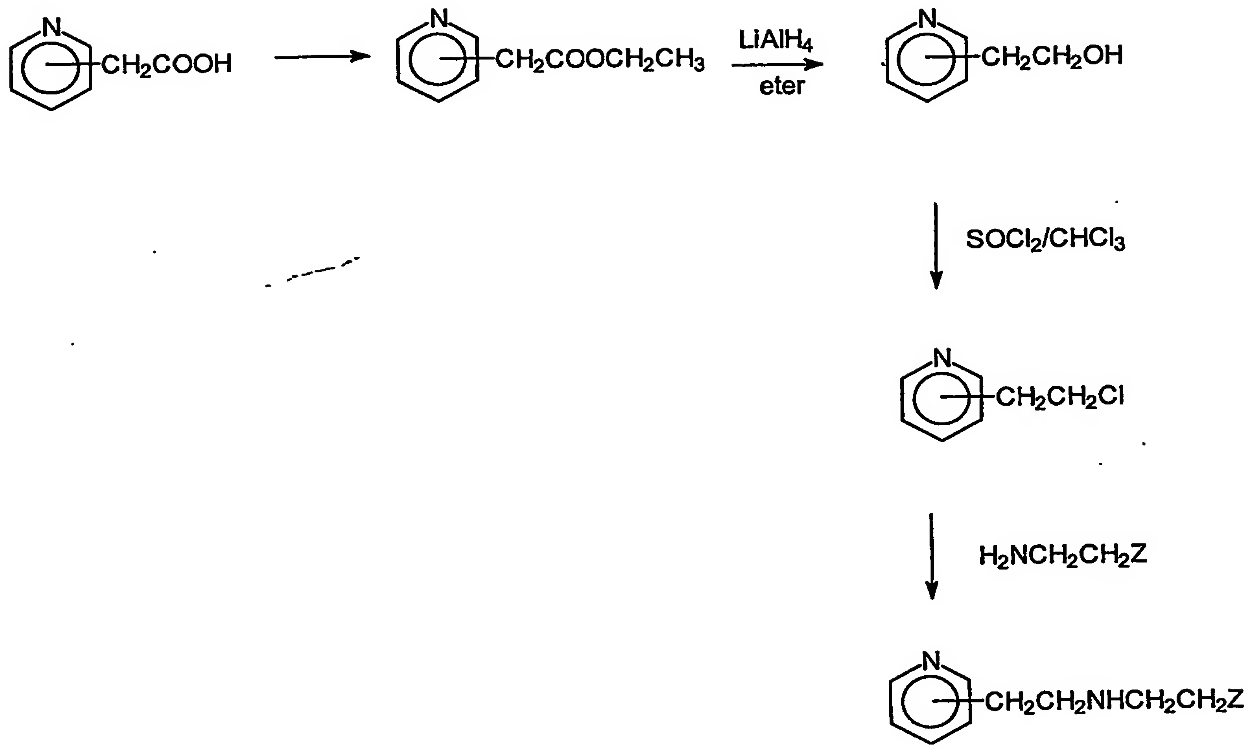
V zadnji stopnji sinteze karbonilno skupino v novih intermediarnih spojinah XI reduciramo v alkoholno skupino. Reakcijo lahko izvedemo z običajnimi redukcijskimi sredstvi, prednostno tistimi primernimi za redukcijo karbonilne skupine v skupini $-R_2HN-CO-$. Zlasti primeren je kompleksni kovinski hidrid, kot $LiAlH_4$ v inertnem topilu, prednostno etru, kot tetrtahidrofuranu (THF), dietil etru, dioksanu in podobno. Pri tem dobljene želene naslovne piridiletanol (feniletil) amine s formulo I izoliramo in očistimo z običajnimi metodami, najbolje s kolonsko kromatografijo na kremeničnem gelu in jih nato po želji presnovimo v njihove fiziološko sprejemljive kislinske adicijske soli.

Postopka za pridobivanje novih derivatov piridiletanol (feniletil) amina s formulo I v skladu z variantama (a) in (b) sta ponazorjena na sliki 5.

Sintezo novih derivatov piridil (feniletil) aminov s formulo I, kjer pomeni R_1 vodikov atom lahko izvedemo tako, da nove spojine v smislu izuma s formulo I, kjer R_1 pomeni hidroksilno skupino, najprej acetiliramo na običajen način, kot z acetanhidridom, ter nato dobljeno O-acetilno spojino katalitsko hidrogeniramo po znanih metodah, kot s paladijem na nosilcu, na primer barijevem sulfatu, po sledeči varianti c)



Po drugi varianti lahko pripravimo nove derivate piridiletanol (feniletil) amina s formulo I, kjer R_1 pomeni vodikov atom, po sledeči varianti d)



Izhodno na mestih 2, 3 ali 4 - substituirano piridil ocetno kislino zaestrimo na običajen način poznan v tehniki, na primer s presnovo v njen etilni ester piridil ocetne kisline, ki ga nato reduciramo z običajnimi redukcijskimi sredstvi, prednostno tistimi za redukcijo esterske skupine v alkoholno skupino. Zlasti primeren je kompleksni kovinski hidrid, kot litijev aluminijev hidrid ($LiAlH_4$) v inertnem topilu, prednostno etru, kot dietil etru, tetrahidrofuranu, dioksanu in podobno. Pri tem dobljeni na mestu 2, 3 ali 4-substituiranem piridil etanolu presnovimo v na mestu 2, 3 ali 4-substutuiran piridil etilenklorid z običajnimi klorimimi sredstvi, na primer s tionil kloridom v inertnem topilu, kot kloroformu. Z dobljenim substituiranim piridiletilenkloridom alkiliramo primarne amine s formulo VI do naslovnih derivatov piridiletanol (fenetil) aminov s formulo I, kjer R_1 pomeni vodikov atom.

V skladu s smotrom izuma smo ugotavljali vpliv novih derivatov piridiletanol (feniletil) aminov kot ligandov sigma receptorjev v inhibiciji biosinteze holesterola. Uporabili smo *ex vivo* metodo metaboličnega označevanja celičnih linij človeških nesmrtnih hepatocitov. Celicam smo dodali radioaktivno označeni predhodnik holesterola [^{3}H] acetat, z ali brez dodatka sigma ligandov. Izvedena sta bila dva medsebojno neodvisna poiskusa metaboličnega označevanja in analize sterolov. Rezultati, pridobljeni v obeh poiskusih, so ponovljivi in kažejo, da testirane substance znatno inhibirajo sintezo holesterola.

Izmed novih ligandov sigma receptorjev v smislu izuma je največje inhibiranje sinteze holesterola pokazala spojina 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol, v obliki dihidrobromidne soli (oznaka BK 35 . 2 HBr).

Znano je namreč, da se sigma ligandi vežejo na sigma receptorje, ki so strukturni homologi sterolne Δ8,7 - izomeraze, ker spadajo v isto gensko družino. Sterolna Δ8,7-izomeraza nastopi v poznih stopnjah biosinteze holesterola, kot ponazorjeno na sliki 1. Iz slike 1 je razvidno, da sta najpogostejša substrata Δ8-holestenol in cimosterol, ki se med seboj razlikujeta v nasičenosti stranske verige na položaju Δ24,25. Na sliki 2 je ponazorjena biosinteza holesterola z označenimi prijemališči inhibitorjev holesterola.

Tako je vpliv novih piridiletanol (feniletil) aminov kot sigma ligandov v smislu izuma bolj selektiven od vpliva v terapiji uporabljanih statinov, kot lovastatina ali pravastatina, ki inhibirajo HMG CoA reduktazo, ki nastopi v zgodnji fazi biosintetske poti holesterola. Z inhibicijo sinteze holesterola na zadnjih stopnjah biosintetske poti lahko z novimi piridiletanol (feniletil) amini v smislu izuma dosežemo bolj ciljno delovanje in z manj stranskimi učinki, zato so te spojine zlasti primerne za zdravljenje hiperholesterolemije in hiperlipemije. Takšno delovanje novih piridiletanol (feniletil) aminov v smislu izuma je bilo resnično nepričakovno, ker v medicini in terapiji nasploh še niso uporabljane učinkovine, ki bi zniževale nivo holestrerola na zadnjih stopnjah biosintetske poti holesterola.

Z uporabo novih piridiletanol (feniletil) aminov s formulo I v smislu izuma pri pacientih s patološko povišano biosintezo holesterola se nivo holesterola v krvi teh pacientov znatno zniža. Odmerek in pogostost uporabe so odvisni tako od lastnosti posamezne spojine, njene biouporabnosti, farmakokinetične lastnosti ter stanja pacientov.

Farnacevtski pripravki vsebujejo učinkovino skupaj s fiziološko sprejemljivim organskim ali anorganskim nosilcem, kot na primer vodo, laktozo, škrob in derivate škroba, magnezijev stearat, smukec, rastlinska olja in podobno. Farmacevtski pripravki se prednostno uporabljajo oralno, na primer v obliki tablet, kapsul, pilul, praškov, granulatov, raztopin, sirupov, suspenzij, eliksirjev in podobno. Uporaba je lahko tudi parenteralna, na primer v obliki sterilnih raztopin, suspenzij ali emulzij. Farmacevtski pripravki se lahko sterilizirajo in / ali vsebujejo sestavine, kot prezervative, stabilizatorje, emulgatorje, puferne substance, in druge dodatke.

Izum opisujejo, vendar z ničemer ne omejujejo sledeči primeri:

PRIMER 1

1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino)etanol (BK 31)

Priprava izhodnih spojin:

N-propil-(β-feniletil)amin

V bučko nalijemo 1.1 ml (9.5 mmol) feniletilamina, 0.8 ml (9.5 mmol) n-propiljodida, 5 ml trietilamina in 5 ml THF (tetrahidrofuran) ter reakcijsko zmes segrevamo pri temperaturi refluksa reakcijske zmesi 3.5 ure. Nato reakcijsko zmes ohladimo, odfiltriramo pri tem izločeno sol, raztopino uparimo in želeno spojino očistimo s kolonsko kromatografijo na kremeničnem gelu (silikagelu) (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 : 3). Dobimo 0.62 g (40%) N-propil-(β-feniletil)amina v obliki olja (molska masa: 163.264, formula: $C_{11}H_{17}N$).

3-bromoacetilpiridin hidrobromid

K 10 g (82.5 mmol) 3-acetilpiridina dolijemo 30 ml 48%-ne bromovodikove kisline. Reakcijsko zmes segrejemo do 70°C in ji med mešanjem počasi dokapavamo 4.2 ml broma. Po končanem dodajanju broma reakcijsko zmes mešamo še 15 minut pri isti temperaturi, nato reakcijsko zmes ohladimo na ledu, odfiltriramo pri tem izločeno kristalinično spojino in dobro speremo z acetonom. Dobimo 21 g (90%) 3-bromoacetilpiridin hidrobromida, ki se tali pri 195-200°C.

Priprava naslovnega 1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino)etanola

K 1.01 g (3.6 mmol) 3-bromoacetilpiridin hidrobromida dolijemo 20 ml abs. etanola in dodamo 0.5 g (13.2 mmol) natrijevega borhidrida. Reakcijsko zmes mešamo pri temperaturi 20°C dve uri. Nato reakcijsko zmes filtriramo in filtratu, ki vsebuje 3-piridiloksiran, dolijemo 0.96 g (5.9 mmol) N-propil-(β-feniletil)amina. Reakcijsko zmes segrevamo pri temperaturi refluksa reakcijske zmesi 4 ure. Nato reakcijsko zmes uparimo do suhega preostanka, ki mu dolijemo 20 ml kloroforma, trden del odfiltriramo, filtrat uparimo in pri tem dobljen oljnat preostanek očistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 : 3). Dobimo 0.56 g (55%) naslovne spojine v obliki olja.

0.56 g (2 mmol) očiščene oljnate baze 1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino) etanola raztopimo v 5 ml acetona. Dobljeno raztopino hladimo na ledu in ji med mešanjem dodamo 2.5 ml etanolne raztopine bromovodikove kisline (0.35 g (4.3 mmol HBr)). Pri tem se izloči oborina, ki ji dolijemo še 3 ml dietil etra. Po 2 urah mešanja na ledu odfiltriramo kristaliničen produkt, ki ga speremo z dietil etrom. Dobimo 0.7 g (80%) 1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino)etanol dihidrobromida, ki se tali pri 113 - 120°C (molska masa: 446.238, bruto formula: $C_{18}H_{24}N_2O$. 2HBr).

H^1 NMR spekter, D_2O, ppm glede na DSS (0ppm): 8.89, 8.80 (2H), 8.65, 8.57 (1H), 8.10 (1H), 7.38 (5H), 5.47 (1H), 3.7-3.1 (8H), 1.80 (2H), 0.97 (3H).

IR (infra-rdeči) spekter (KBr ploščica) je ponazorjen na sliki 6.

PRIMER 2

1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanol (BK 33)

Priprava 1-(3-piridil)-2-metilaminoetanola

K 1.01 g (3.6 mmol) 3-bromoacetilpiridin hidrobromida, pripravljenega v skladu s primerom 1, dolijemo 20 ml abs. etanola in dodamo 0.5 g (13.2 mmol) natrijevega borhidrida ter reakcijsko zmes mešamo pri temperaturi 20°C dve uri. Nato reakcijsko zmes filtriramo in filtratu, ki vsebuje 3-piridiloksiran, dolijemo 1.3 ml 33%-ne etanolne raztopine metilamina. Reakcijsko zmes segrevamo pri temperaturi refluksa reakcijske zmesi 5 ur. Potem reakcijsko zmes uparimo do suhega preostanka, ki mu dolijemo 20 ml kloroforma, trden del odfiltriramo, filtrat uparimo in pri tem dobljen oljnat preostanek očistimo s kolonsko kromatografijo na kremeničnem gelu (mobilna faza: $CHCl_3$: CH_3OH = 10 : 3). Dobimo 0.33 g (60%) naslovne spojine v obliki oljnate baze (molska masa: 152.196, bruto formula: $C_8H_{12}N_2O$).

Priprava 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)acetil-N-metilamino)etanola

V bučko zatehtamo 542 mg (2.6 mmol) DCC (dicikloheksilkarbodiimid) in dolijemo 2 ml metilen klorida (CH_2Cl_2) ter nato med mešanjem dokapavamo raztopino 538 mg (2.6 mmol) 3,4-diklorofenil ocetne kisline v 3 ml metilen klorida, pri čemer se izloči oborina. Nato reakcijsko zmes mešamo 5 minut ter ji dodamo 400 mg (2.6 mmol) 1-(3-piridil)-2-metilaminoetanola. Reakcijsko zmes mešamo nato še 1 uro pri temperaturi 20°C. Pri tem izločeno oborino odfiltriramo in dobljeno raztopino uparimo. Uparjen filtrat čistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 : 0,5). Dobimo 715 mg (80%) 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)acetil-N-metilamino)etanola (molska masa: 339.224, bruto formula: $C_{16}H_{16}N_2O_2Cl_2$)

Priprava naslovnega 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil-N-metilamino)etanola (BK-33)

V bučko zatehtamo 0.53 g (13.9 mmol) litijevegaaluminijevega hidrida ($LiAlH_4$) in dolijemo 6 ml brezvodnega tetrahidrofurana (THF) in zmes hladimo na ledu. Nato reakcijski zmesi med mešanjem po kapljicah dodajamo raztopino 1.1 g (3.2 mmol) 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-etilamino)etanola v 10 ml

brezvodnega tetrahidrofurana (THF). Po končanem dodajanju reakcijsko zmes mešamo še 1 uro pri temperaturi 20°C. Nato reakcijsko zmes ohladimo na ledu in ji nato med intenzivnim mešanjem postopoma dodamo 6.5 ml 15%-nega NaOH in dolijemo 16 ml metilen klorida (CH_2Cl_2). Organsko fazo ločimo, jo posušimo z Na_2SO_4 in uparimo na rotavaporju do oljnatega preostanka, ki ga očistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: CH_3OH : etil acetat = 10 : 2). Dobimo 0.63 g (60%) naslovne spojine v obliki oljnate baze.

0.60 g (1.84 mmol) očiščene oljnate baze raztopimo v 3.5 ml acetona. Raztopino hladimo na ledu in med mešanjem dodamo 2.4 ml etanolne raztopine bromovodikove kisline (0.328 g HBr; 4.1 mmol). Pri tem se izloči oborina, ki ji dolijemo še 2 ml dietil etra. Po 2 urah mešanja reakcijske zmesi na ledu odfiltriramo pri tem dobljeni kristaliničen produkt, ki ga speremo z dietil etrom. Dobimo 0.76 g (80%) 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-metilamino) etanol dihidrobromida, ki se tali pri 157 - 161°C (molska masa: 487.074, bruto formula: $C_{17}H_{18}N_2OCl_2 . 2HBr$).

H^1 NMR spekter; D_2O, ppm glede na DSS (0ppm): 8.90 (1H), 8.78 (1H), 8.64 (1H), 8.10 (1H), 7.50 (2H), 7.24 (1H), 5.50 (1H), 3.52 (4H), 3.08 (5H).

IR spekter (KBr ploščica) je ponazorjen na sliki 9.

PRIMER 3

1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol (BK-35)

Priprava 1-(3-piridil)-2-propilaminoetanola

K 1.01 g (3.6 mmol) 3-bromoacetilpiridin hidrobromida, pripravljenega v skladu s primerom 1, dolijemo 20 ml absolutnega etanola in dodamo 0.5 g (13.2 mmol) natrijevega borhidrida ($NaBH_4$). Reakcijsko zmes mešamo 2 uri pri temperaturi 20°C. Nato reakcijsko zmes filtriramo in filtratu, ki vsebuje 3-piridiloksiran, dolijemo 0.7 ml n-propilamina. Reakcijsko zmes nato segrevamo pri temperaturi refluksa reakcijske zmesi 5 ur. Nato reakcijsko zmes uparimo do suhega preostanka, ki mu dolijemo 20 ml kloroforma, trden del odfiltriramo, filtrat uparimo in oljnat preostanek čistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: CH_3OH : etil acetat = 10 : 2). Dobimo 0.33 g (50%) naslovne spojine v obliki oljnate baze (molska masa: 367.278, bruto formula: $C_{18}H_{20}N_2O_2Cl_2$)

Priprava 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-propilamino)etanola

V bučko zatehtamo 630 mg (3.1 mmol) DCC (dicikloheksilkarbodiimida) in dolijemo 3 ml metilen klorida ter med mešanjem dokapavamo raztopino 625 mg (3.1 mmol) 3,4-diklorofenil ocetne kisline v 5 ml metilen klorida, pri čemer se izloči

oborina. Nato reakcijsko zmes mešamo 5 minut ter ji dodamo raztopino 550 mg 1-(3-piridil)-2-propilaminoetanola v 6 ml metilen klorida. Reakcijsko zmes nato mešamo še 1 uro pri temperaturi 20°C, pri tem izločeno oborino odfiltriramo in dobljeno raztopino uparimo. Uparjen filtrat čistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 . 0,5). Dobimo 0.56 g (50%) 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-propilamino)etanola v obliki olja (molska masa: 367.278, bruto formula: $C_{18}H_{20}N_2O_2Cl_2$).

Priprava naslovnega 1-(3-piridil)-2-[N-(2-(3,4-diklorofenil)etil)-N-propilamino]etanola (BK-35)

V bučko zatehtamo 0.43 mg (11.4 mmol) litijevegaaluminijevega hidrida ($LiAlH_4$) in dolijemo 6 ml brezvodnega tetrahidrofurana (THF) in zmes hladimo na ledu. Nato med mešanjem po kapljicah dodajamo raztopino 1 g 1-(3-piridil)-2-(N-2-(3,4-diklorofenil)acetil)-N-propilamino)etanola v 10 ml brezvodnega THF. Po končanem dodajanju reakcijsko zmes mešamo še 1 uro pri temperaturi 20°C. Nato reakcijsko zmes ohladimo na ledu in ji med intenzivnim mešanjem postopoma dodamo 6.4 ml 15%-nega NaOH in dolijemo 16 ml metilen klorida. Organsko fazo ločimo, jo sušimo z Na_2SO_4 in uparimo na rotavaporju. Uparjen preostanek očistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, prva mobilna faza: $CHCl_3$: CH_3OH = 10 : 0,5 ; druga mobilna faza: etil acetat : CH_3OH = 10 : 1,5). Dobimo 0.58 g (60%) naslovne spojine v obliki oljnate baze.

0.50 g (1.4 mmol) dobljene očiščene oljnate baze raztopimo v 4 ml acetona. Dobljeno raztopino hladimo na ledu in ji med mešanjem dodamo 1.1 ml etanolne raztopine bromovodikove kisline (0.25 g HBr; 3.1 mmol). Pri tem se izloči bela oborina, ki ji dolijemo še 3 ml dietil etra in po 2 urah mešanja na ledu odfiltriramo kristaliničen produkt, ki ga spreremo z dietil etrom. Dobimo 0.62 g (85%) 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol dihidrobromida, ki se tali pri 136 - 139°C (molska masa: 515.124; bruto formula: $C_{18}H_{22}N_2OCl_2$. 2HBr)

H^1 NMR spekter, D_2O, ppm glede na DSS (0ppm): 8.56 (2H), 8.01 (1H), 7.59 (1H), 7.50 (2H), 7.23 (1H), 5.25 (1H), 3.50 (4H), 3.30 (2H), 3.11 (2H), 1.78 (2H), 0.96 (3H)

IR spekter (KBr ploščica) je ponazorjen na sliki 8.

PRIMER 4

1-(4-piridil)-2(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanol (BK-38)

Priprava 1-(4-piridil)-2-metilaminoetanola

K 1.01 g (3.6 mmol) 4-bromoacetilpiridin hidrobromida, pripravljenega v skladu s primerom 1, dolijemo 20 ml absolutnega etanola in dodamo 0.5 g (13.2 mmol) natrijevega borhidrida ter reakcijsko zmes mešamo 2 uri pri temperaturi 20°C.

Nato reakcijsko zmes filtriramo in filtratu, ki vsebuje 4-piridiloksiran, dolijemo 1.3 ml 33%-ne etanolne raztopine metilamina. Nato reakcijsko zmes segrevamo pri temperaturi refluksa reakcijske zmesi 3 ure. Nato reakcijsko zmes uparimo do suhega preostanka, ki mu dolijemo 20 ml kloroforma, trden del odfiltriramo, filtrat uparimo in oljni preostanek očistimo s kolonsko kromatografijo na kremeničnem gelu (silikagel 60, mobilna faza: CH_3OH : etil acetat = 10 : 2). Dobimo 0.30 g (55%) naslovne spojine v obliki oljnate baze (molska masa: 152.196; bruto formula: $C_8H_{12}N_2O$).

Priprava 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-metilamino)etanola

V bučko zatehtamo 0.54 g (2.6 mmol) DCC (dicikloheksilkarbodiimida) in dolijemo 2 ml metilen klorida ter nato med mešanjem dokapavamo raztopino 0.54 g (2.6 mmol) 3,4-diklorofenil ocetne kisline v 4 ml metilen klorida, pri čemer se izloči oborina. Reakcijsko zmes mešamo 5 minut ter ji nato dodamo raztopino 400 mg (2.6 mmol) 1-(4-piridil)-2-metilaminoetanola v 3 ml metilen klorida. Reakcijsko zmes mešamo še 1 uro pri temperaturi 20°C. Pri tem izločeno oborino odfiltriramo in dobljeno raztopino uparimo. Uparjen filtrat čistimo s kolonsko kromatografijo na kremeničnem gelu (silikagelu) (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 : 0,5). Dobimo 0.53 g 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-metilamino)etanola.

Priprava naslovnega 1-(4-piridil)-2-[(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanola (BK-38)

V bučko zatehtamo 510 mg (13.5 mmol) litijevegaaluminijevega hidrida ($LiAlH_4$) in dolijemo 6 ml brezvodnega THF ter zmes ohladimo na ledu. Nato med mešanjem po kapljicah dodajamo raztopino 1.02 g (3 mmol) 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)acetil)-N-metilamino)etanola v 10 ml brezvodnega THF. Po končanem dodajanju reakcijsko zmes mešamo še 1 uro pri sobni temperaturi. Nato reakcijsko zmes ohladimo na ledu in ji nato med intenzivnim mešanjem postopoma dodamo 6.6 ml 15%-nega NaOH ter dolijemo 16 ml metilen klorida. Organsko fazo ločimo, jo posušimo z Na_2SO_4 in uparimo na rotavaporju do oljnatega preostanka, ki ga čistimo s kolonsko kromatogfrafijo na silikagelu (silikagel 60, mobilna faza: $CHCl_3$: CH_3OH = 10 : 1). Dobimo 0.67 g (85%) 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-metilamino)etanol dihidrobromida, ki se tali pri 191 - 194°C (molska masa: 484.074; bruto formula: $C_{17}H_{18}N_2OCl_2 . 2HBr$).

H1 NMR spekter, D2O, ppm glede na DSS (0ppm): 8.81 (2H), 8.14 (2H), 7.47 (2H), 7.22 (1H), 5.54 (1H), 3.50 (4H), 3.08 (5H)

IR spekter (KBr ploščica) je ponazorjen na sliki 7.

PRIMER 5

Testiranje štirih ligandov sigma receptorjev (BK-31 . 2 HBr, BK-33 . 2 HBr, BK-35 . 2 HBr in BK-38 . 2 HBr) iz primerov 1 do 4, inhibitorjev biosinteze holesterola na stopnji sterolne Δ7,8 – izomeraze

Ugotavljali smo vpliv štirih novih ligandov sigma receptorjev (BK-31 . 2 HBr, BK-33 . 2 HBr, BK-35 . 2 HBr in BK-38 . 2 HBr), pripravljenih v skladu s primeri 1 do 4, na inhibicijo sintezo holesterola. Uporabili smo *ex vivo* metodo metaboločnega označevanja celičnih linij človeških nesmrtnih hepatocitov. Celicam smo dodali radioaktivno označen prekurzor holesterola [^{3}H] acetat, z ali brez dodatka ligandov. Izvedli smo dva medsebojno neodvisna poiskusa metaboličnega označevanja in analize sterolov.

Materiali in metode

Gojenje celic z dodatkom ligandov sigma receptorjev

Trajno človeško celično linijo jetrnih celic HepG$_2$ smo precepili v stekleničke s površino 75 cm^2 v razmerju 1:2, dve steklenički na eksperimentalni pogoj. Celice smo gojili v gojišču DMEM (L-arginin.HCL 0.084 g/l, L-cistin.2HCl 0.0626 g/l, L-glutamin 0.584 g/l, glicin 0.03 g/l, L-histidin.HCl.H_2O 0.042 g/l, L-izoleucin 0.105 g/l, L-leucin 0.105 g/l, L-lizin.HCl 0.146 g/l, L-metionin 0.03 g/l, L-fenilalanin 0.066 g/l, L-serin 0.042 g/l, L-treonin 0.095 g/l, L-triptofan 0.016 g/l, L-tirosin 2Na.2H_2O 0.10379 g/l, L-valin 0.094 g/l, holin klorid 0.004 g/l, folna kislina 0.004 g/l, mio-inozitol 0.0072 g/l, niacinamid 0.004 g/l, D-pantotenska kislina 0.004 g/l, piridoksal.HCl 0.004 g/l, riboflavin 0.0004 g/l, tiamin.HCl 0.004 g/l, kalcijev klorid . 2H_2O 0.265 g/l, feri nitrat.9 H_2O 0.0001 g/l, magnezijev sulfat [brezvodni] 0.09767 g/l, kalijev klorid 0.4 g/l,. natrijev klorid 6.4 g/l, natrijev fosfat monobazičen [brezvodni] 0.109 g/l, glukoza 4.5 g/l in fenol rdeče.Na 0.0159 g/l) s 5% telečjim serumom in 1% L-glutaminom. Po 24 urah smo celicam dodali gojišče s 100 μM koncentracijo ligandov sigma receptorjev (BK-31 . 2 HBr, BK-33 . 2 HBr, BK-35 . 2 HBr in BK-38 . 2 HBr). Kot pozitivno kontrolo inhibicije sinteze holesterola smo uporabili gojišče z znanimi inhibitorji sinteze holesterola: 100 μM koncentracije lovastatina ali pravastatina, ki sta oba inhibitorja HMG CoA reduktaze in 100 μM koncentracije flukonazola, ki je inhibitor encimov naddružine citokromov P450, med katere spada tudi lanosterol 14α - demetilaza (CYP51). Kot negativno kontrolo smo celice gojili v osnovnem gojišču brez dodatka inhibitorjev. Po 24 urah smo gojišče zamenjali. Po 48 urah smo dodali [^{3}H] acetat v koncentraciji 40 μCi na 1 ml gojišča (400 μCi na stekleničko). Po petih urah smo gojišče aspirirali, celice tripsinizirali z 2 ml tripsina, zbrali v 4 medija, celice odcentrifugirali in celični pelet raztopili v destilirani vodi (1 ml na stekleničko). Celice smo homogenizirali z zamrzovanjem in odtajanjem. Iz homogenata smo ekstrahirali sterole. V homogenatu smo določili koncentracijo proteinov z Bio-rad-ovim reagentom za določevanje proteinov po navodilih tega proizvajalca.

Ekstrakcija sterolov

Homogenat smo prenesli v steklene viale z inertnim pokrovom, dodali 3 ml ekstrakcijske raztopine (75% n-heptan : 25% izopropanol (vol./vol.)). Zaprte stekleničke smo močno stresali na stresalniku v temnem prostoru 2 uri. Po ekstrakciji smo stekleničke centrifugirali (2000 g, 10 minut), organsko fazo prenesli v steklene epruvete, jo posušili pod tokom dušika, sprali z 2 ml n-heptana HPLC čistote, odcentrifugirali (2000 g, 5 minut) in prenesli v svežo epruveto. Vzorec smo shranili v HPLC topilu do analize na hladnem in temnem.

HPLC analiza
("High performance liquid chromatography"– kromatografija visoke ločljivosti)

Posušeni ekstrakt smo raztopili v 250 µl n-heptana. Alikvote po 100 µl smo injicirali na kolono za normalno fazo (ChromSpher Si, 150 mm x 3 mm, velikosti delcev 5 µm) z mobilno fazo 99,5% n-heptan : 0,5% izopropanol pri pretoku 1 ml / min in sobni temperaturi.

Določevanje sterolov

Sterole smo določili s pomočjo ultravijoličnega detektorja pri dveh valovnih dolžinah: 200 nm za lanosterol / T-MAS in holesterol in 249 nm za 4,4-dimetil-α-holesta-8,14,24-trien-3β-ol (FF-MAS) in interni standard ergosterol. Pri določevanju sterolov po metaboličnem označevanju celic smo uporabili radiodetektor s pretočno celico. Določitev je bila izvedena na osnovi retencijskih časov standardov: lanosterol (Steraloids), holesterol (Steraloids), ergosterol (Sigma), 4,4-dimetil-α-holesta-8,14,24,-trien-3β-ol (FF-MAS) in [^{3}H] FF-MAS (laboratorij A.G.Byskov, Rikhospitalitet, Univerza v Copenhagnu). Rezultate smo normalizirali glede na količino internega standarda ergosterola kot tudi glede na koncentracijo proteinov v homogenatu. Prikazani so kot povprečna vrednost dveh meritev s pripadajočo standardno deviacijo.

Rezultati

Metabolično označevanje celic je pokazalo odlične rezultate na nivoju znižanja količine sintetiziranega holesterola. Za holesterol smo zasledili vrh na radiodetektorju pri okoli 6,0 min. Slika 3 ponazarja količino radioktivno označenega holesterola po metaboličnem označevanju celic in dodatku različnih inhibitorjev. Negativna kontrola, normalni medij – gojišče brez dodatka inhibitorjev, A – analiza 1, B – analiza 2. AU – naključne enote. Vrh holesterola, kot je ponazorjeno na sliki 3, se je pri celicah, gojenih s sigma ligandi, močno znižal, še najbolj pri spojini z oznako BH-35 . 2HBr. Poleg popolnega zavrtja sinteze holesterola, kot je ponazorjeno na sliki 3, so testirane spojine pokazale tudi vpliv na kopičenje

zgodnjih intermediatov poskvalenskega dela biosinteze holesterola. Zaznali smo povečanje koncentracije sterolov, ki ustrezajo lanosterolu oziroma 4,4-dimetil-α-holesta-8(9),24,-dien-3β-olu (T-MAS). Največji vpliv je tudi s tega vidika pokazala spojina z oznako BK-35 . 2HBr, kjer smo zaznali desetkratno povečanje količine intermediata, kot je ponazorjeno na sliki 4. Slika 4 ponažarja količino radioaktivnega intermediata sterola X, ki se eluira za holesterolom (7-dehidroholesterol ali latosterol). Oznake so enake kot na sliki 3. A – analiza, B – analiza 2.

Vse analize potrjujejo, da novi derivati piridil (feniletil) aminov kot sigma ligandov v smislu izuma blokirajo sintezo holesterola najverjetneja na stopnji sterol Δ8,7-izomeraze. Ob prisotnosti spojine z oznako BK-35 . 2HBr se količina holesterola zmanjša in poveča se količina intermediatov, ki se nahajajo v biosintezni poti pred sterol Δ8,7-izomerazo.

Kot pozitivna kontrola zanesljivosti analize so služili poiskusi v celicah, gojenih z dobro poznanimi inhibitorji HMG-CoA-reduktaze (lovastatin in pravastatin) in lanosterol 14α-demetilaze (flukonazol). Pri lovastatinu in pravastatinu je bila biosinteza popolnoma zavrta, kot je ponazorjeno na sliki 3. Pri flukonazolu je bila inhibicija sinteze holesterola slabša, kar je pričakovano, saj flukonazol ni specifični inhibitor človeške lanosterol-14α demetilaze. Količina lanosterola oziroma 4,4-dimetil-α-holesta-8(9),24,-dien-3β-ola (T-MAS) pa se pri statinih ni povečala, saj te spojine inhibirajo biosintezo na stopnji HMG-CoA reduktaze, ki je na začetku zaporedja biosinteze holesterola, torej pred lanosterolom in T-MAS.

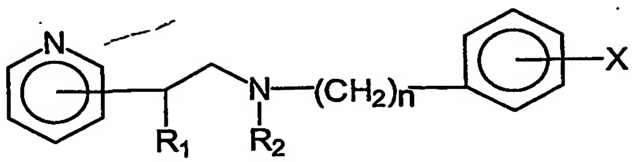
Zaključki

Ugotovili smo, da se je v celicah, gojenih v gojišču z dodatkom testiranih spojin z oznakami BK-31 . 2 HBr, BK-33 . 2 HBr, BK-35 . 2 HBr in BK-38 . 2 HBr, signifikantno zmanjšala količina nastalega holesterola. Na osnovi tega ugotavljamo, da so vse testirane spojine oziroma novi derivati piridiletanol (feniletil) aminov v smislu izuma inhibitorji biosinteze holesterola najverjetneje na stopnji sterolne Δ7,8-izomeraze. Največje zmanjšanje holesterola smo ugotovili pri spojini z oznako BK-35 . 2 HBr, to je 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil)-N-propilamino)etanol dihidrobromid. Rezultati, pridobljeni v dveh neodvisnih poiskusih so skladi in kažejo, da je med vsemi testiranimi sigma ligandi spojina z oznako BK-35 . 2 HBr najboljši inhibitor biosinteze holestrerola v smislu izuma in zato zlasti primeren za zdravljenje hiperholesterolemije in hiperlipemije.

LEK farmacevtska družba d. d.

PATENTNI ZAHTEVKI

1. Spojine s formulo I



I

kjer pomeni

n je celo število od 1 do 4
R_1 je vodikov atom, hidroksilna skupina ali nižja C_{1-6} alkoksi skupina
R_2 je vodikov atom, nižja C_{1-6} alkilna skupina z ravno ali razvejeno verigo
X je vodikov atom, fluor, klor, brom, hidroksilna skupina, trifluormetilna skupina, 3,4-di-Cl, 2,4-di-Cl ali nižja C_{1-6} alkoksi skupina,

njihovi enantiomeri, diastereoizomeri ali racemati ali njihove fiziološko sprejemljive kislinske adicijske soli.

2. Spojine po zahtevku 1, kjer pomeni n celo število 2, R_1 hidroksilno skupino, R_2 metilno, etilno, n-propilno, izopropilno, n-butilno ali izobutilno skupino in X vodikov atom ali z dvema atomoma klora disubstituiran fenil na mestih 3 in 4 ali na mestih 2 in 4.

3. Spojine po zahtevkih 1 in 2, kjer je R_1 hidroksilna skupina v RS konfiguraciji.

4. 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil-N-propilamino) etanol in njegova dihidrobromidna sol.

5. 1-(3-piridil)-2-(N-(2-feniletil)-N-propilamino) etanol in njegova dihidrobromidna sol.

6. 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil-N-metilamino) etanol in njegova dihidrobromidna sol.

7. 1-(4-piridil)-2-(N-(2-(3,4-diklorofenil)-N-metilamino) etanol in njegova dihidrobromidna sol.

8. Spojine s formulo I po kateremkoli od zahtevkov 1 do 7 in njihove fiziološko sprejemljive kislinske adicijske soli kot ligandi sigma receptorjev v inhibiciji biosinteze holesterola za zdravljenje hiperholesterolemije in hiperlipidemije pri ljudeh.

9. Farmacevtski pripravki, označeni s tem, da vsebujejo spojino s formulo I po kateremkoli od zahtevkov od 1 do 7 ali njihove fiziološko sprejemljive kislinske adicijske soli.

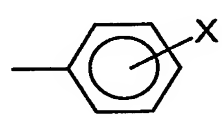
10. Uporaba spojin s formulo I po kateremkoli od zahtevkov 1 do 7 ali njihove fiziološko sprejemljive kislinske adicijske soli kot ligandov sigma receptorjev v inhibiciji biosinteze holesterola za pripravo farmacevtskih pripravkov za zdravljenje hiperholesterolemije in hiperlipemije pri ljudeh.

11. Postopek za pripravo spojin s formulo I po kateremkoli od zahtevkov 1 do 7, označen s tem, da izvedemo

a) alkiliranje sekundarnih aminov s formulo VI

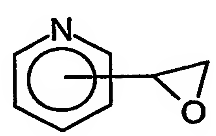
$NHR_2CH_2CH_2Z$ VI

v kateri ima R_2 v formuli I naveden pomen in Z pomeni skupino



v kateri ima X v formuli I naveden pomen,

s piridil oksiranom s formulo VII



VII

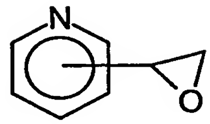
in po želji presnovimo dobljene spojine s formulo I v sol ali

b) alkiliranje primarnih aminov s formulo VIII

R_2NH_2 VIII

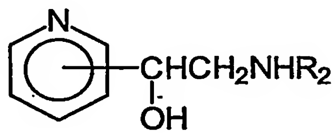
v kateri ima R_2 v formuli I naveden pomen,

s piridil oksiranom s formulo VII



VII

do intermediarnih spojin s formulo IX



IX

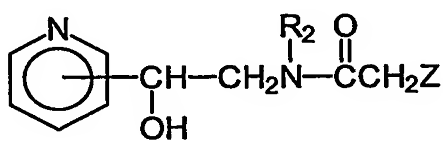
v kateri ima R_2 v formuli I naveden pomen,

ki jih kondenziramo z derivati fenil ocetne kisline s formulo X

$HOOOCH_2CH_2Z$

v kateri ima Z zgoraj naveden pomen,

do intermediarnih spojin s formulo XI



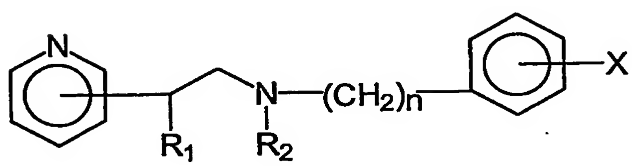
XI

ki jih reduciramo do naslovnih spojin s formulo I, ki jih po želji presnovimo v sol.

LEK: farmacevtska družba d. d.

POVZETEK

Opisani so novi derivati piridiletanol (feniletil) aminov s formulo I



I

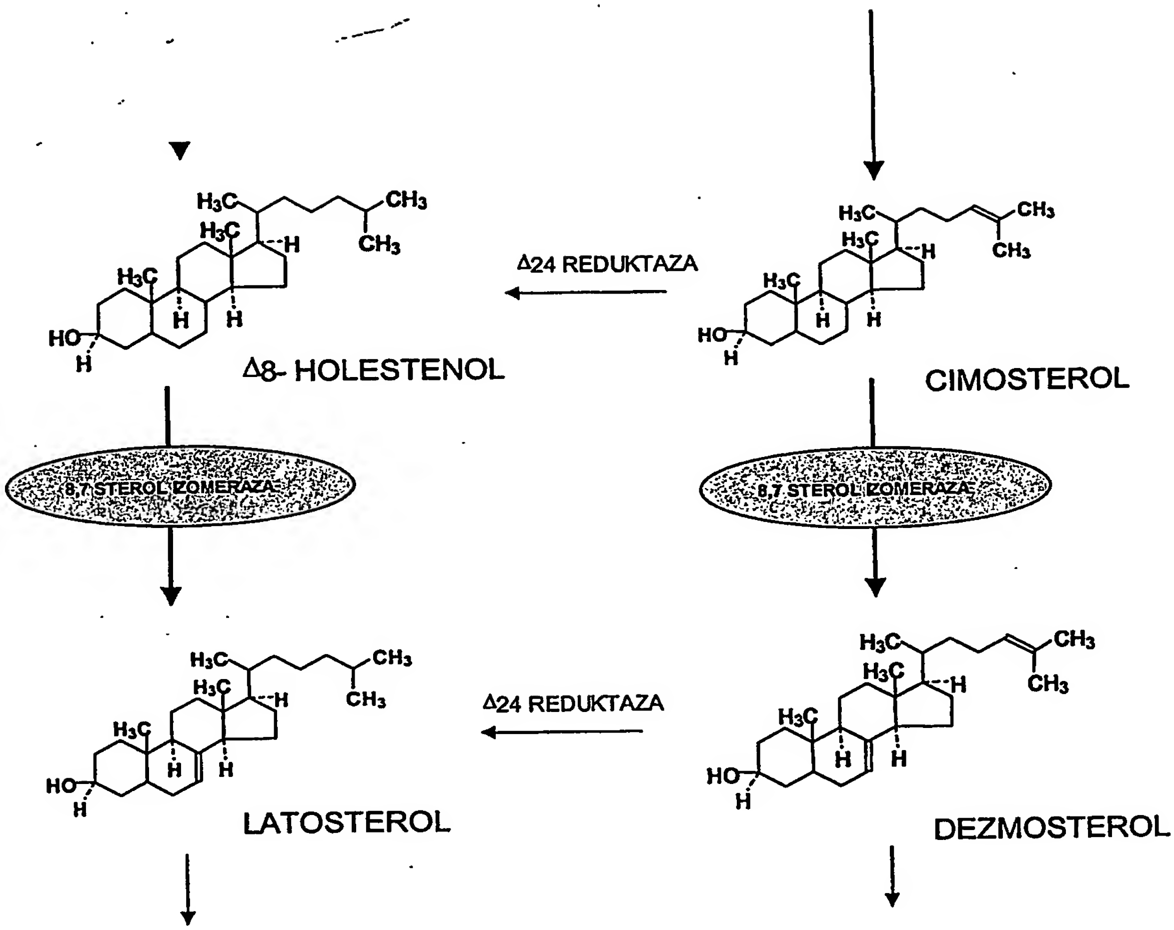
kjer pomeni

n je celo število od 1 do 4
R_1 je vodikov atom, hidroksilna skupina ali nižja C_{1-6} alkoksi skupina
R_2 je vodikov atom, nižja C_{1-6} alkilna skupina z ravno ali razvejano verigo
X je vodikov atom, fluor, klor, brom, hidroksilna skupina, trifluormetilna skupina, 3,4-di-Cl, 2,4-di-Cl ali nižja C_{1-6} alkoksi skupina,

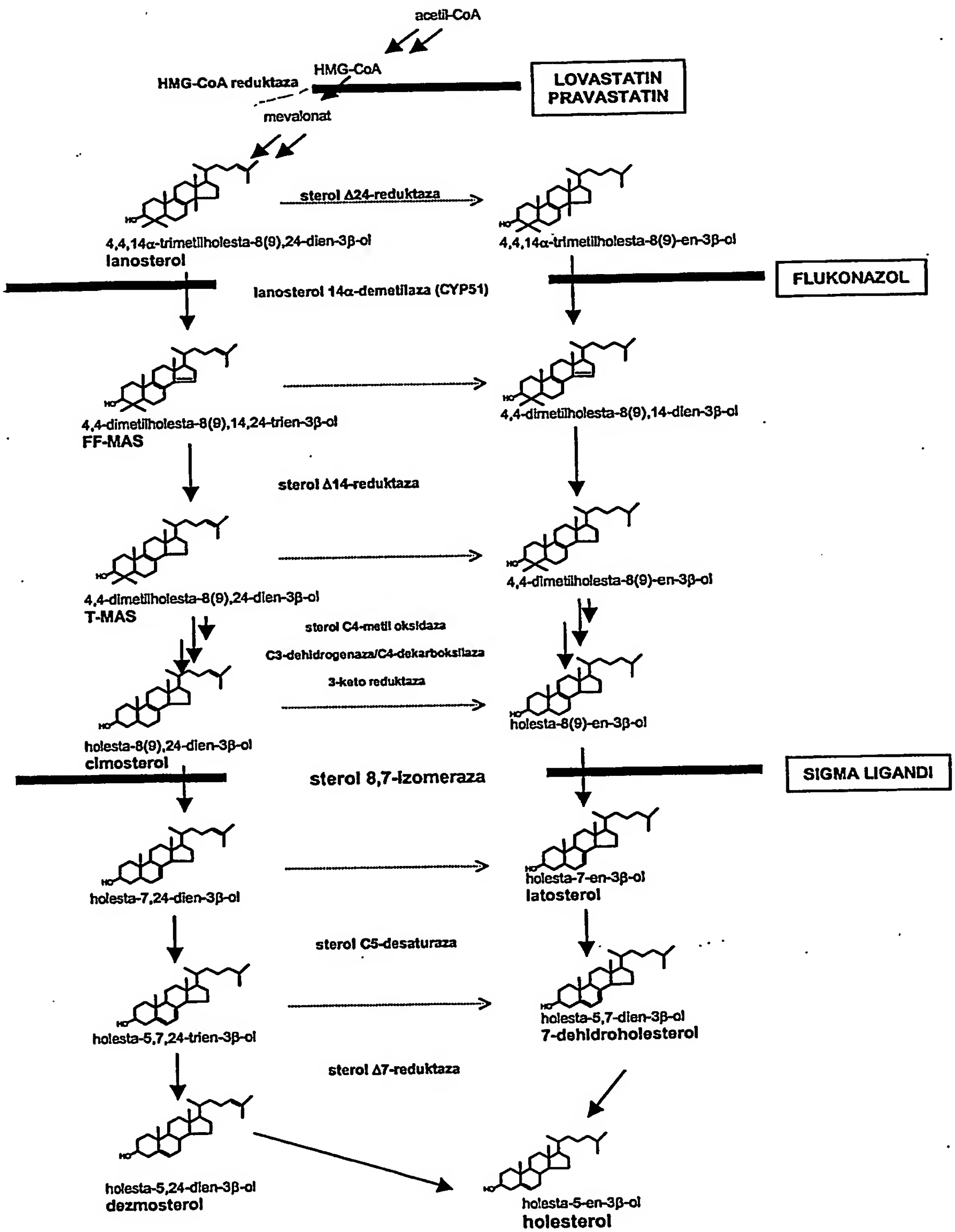
njihovi enantiomeri, diastereoizomeri ali racemati ali njihove fiziološko sprejemljive kislinske adicijske soli, ki so novi ligandi sigma receptorjev v inhibiciji biosinteze holesterola in s tem primerni za zdravljenje hiperholesterolemije in hiperlipidemije pri ljudeh.

Največje zmanjšanje holesterola smo ugotovili pri 1-(3-piridil)-2-(N-(2-(3,4-diklorofenil)etil-N-propilamino) etanolu v obliki dihidrobromidne soli (oznaka BK-35 . 2 HBr).

Slika 1

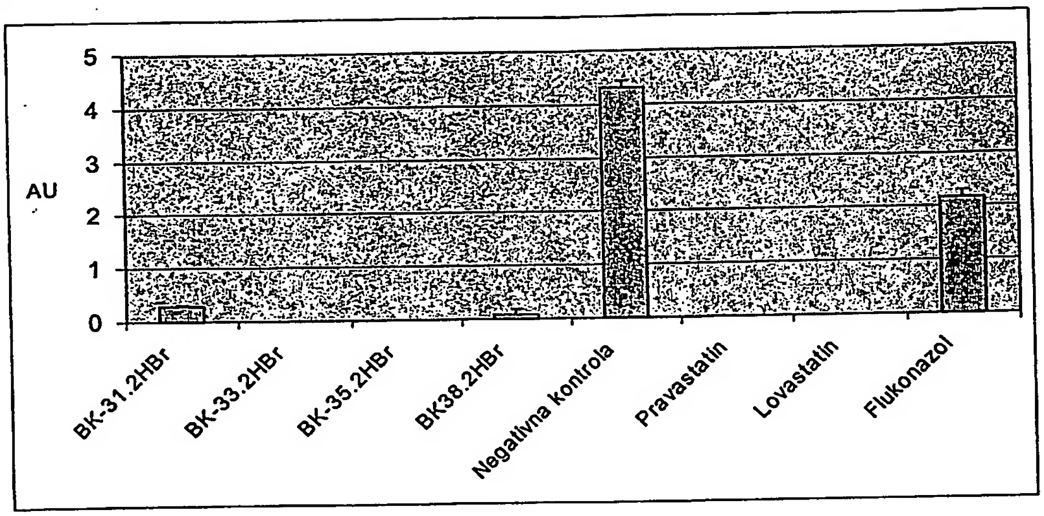

Δ8- HOLESTENOL
Δ24 REDUKTAZA
CIMOSTEROL
8,7 STEROL IZOMERAZA
8,7 STEROL IZOMERAZA
LATOSTEROL
Δ24 REDUKTAZA
DEZMOSTEROL

Slika 2

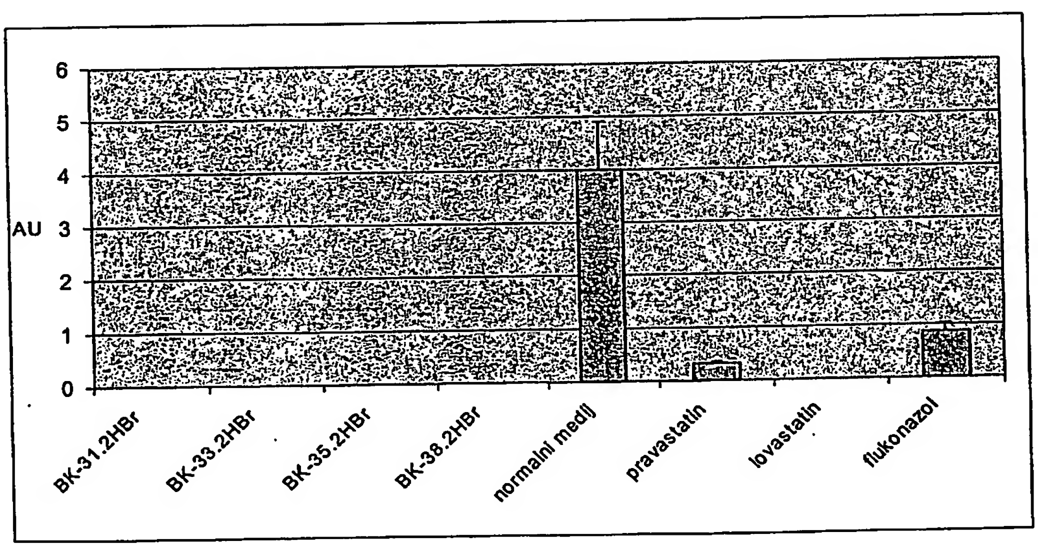

acetil-CoA
HMG-CoA
HMG-CoA reduktaza
mevalonat
LOVASTATIN
PRAVASTATIN
sterol Δ24-reduktaza
4,4,14α-trimetilholesta-8(9),24-dien-3β-ol
lanosterol
4,4,14α-trimetilholesta-8(9)-en-3β-ol
lanosterol 14α-demetilaza (CYP51)
FLUKONAZOL
4,4-dimetilholesta-8(9),14,24-trien-3β-ol
FF-MAS
4,4-dimetilholesta-8(9),14-dien-3β-ol
sterol Δ14-reduktaza
4,4-dimetilholesta-8(9),24-dien-3β-ol
T-MAS
4,4-dimetilholesta-8(9)-en-3β-ol
sterol C4-metil oksidaza
C3-dehidrogenaza/C4-dekarboksilaza
3-keto reduktaza
holesta-8(9),24-dien-3β-ol
cimosterol
holesta-8(9)-en-3β-ol
sterol 8,7-izomeraza
SIGMA LIGANDI
holesta-7,24-dien-3β-ol
holesta-7-en-3β-ol
latosterol
sterol C5-desaturaza
holesta-5,7,24-trien-3β-ol
holesta-5,7-dien-3β-ol
7-dehidroholesterol
sterol Δ7-reduktaza
holesta-5,24-dien-3β-ol
dezmosterol
holesta-5-en-3β-ol
holesterol

Slika 3

A

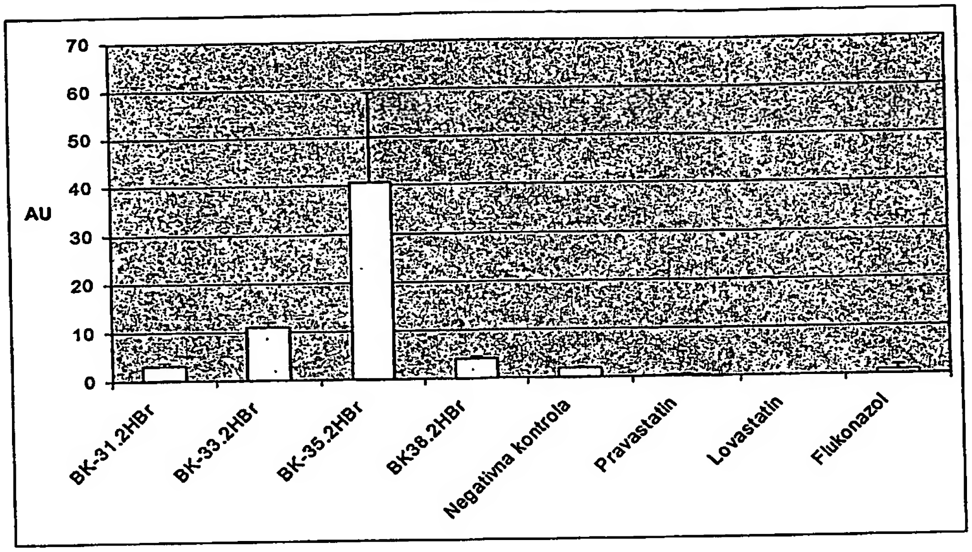

5
4
3
AU
2
1
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK38.2HBr
Negativna kontrola
Pravastatin
Lovastatin
Flukonazol

B

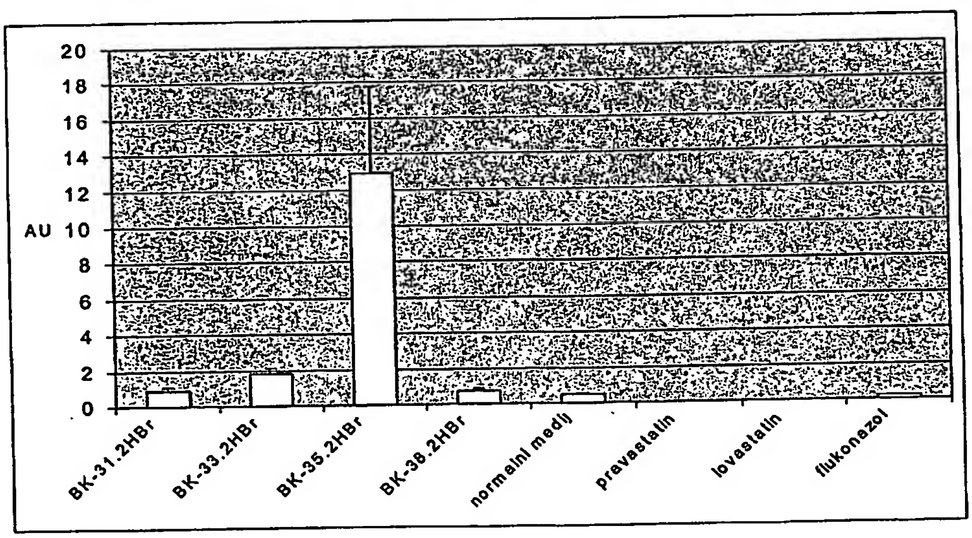

6
5
4
AU
3
2
1
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK-38.2HBr
normalni medij
pravastatin
lovastatin
flukonazol

Slika 4

A

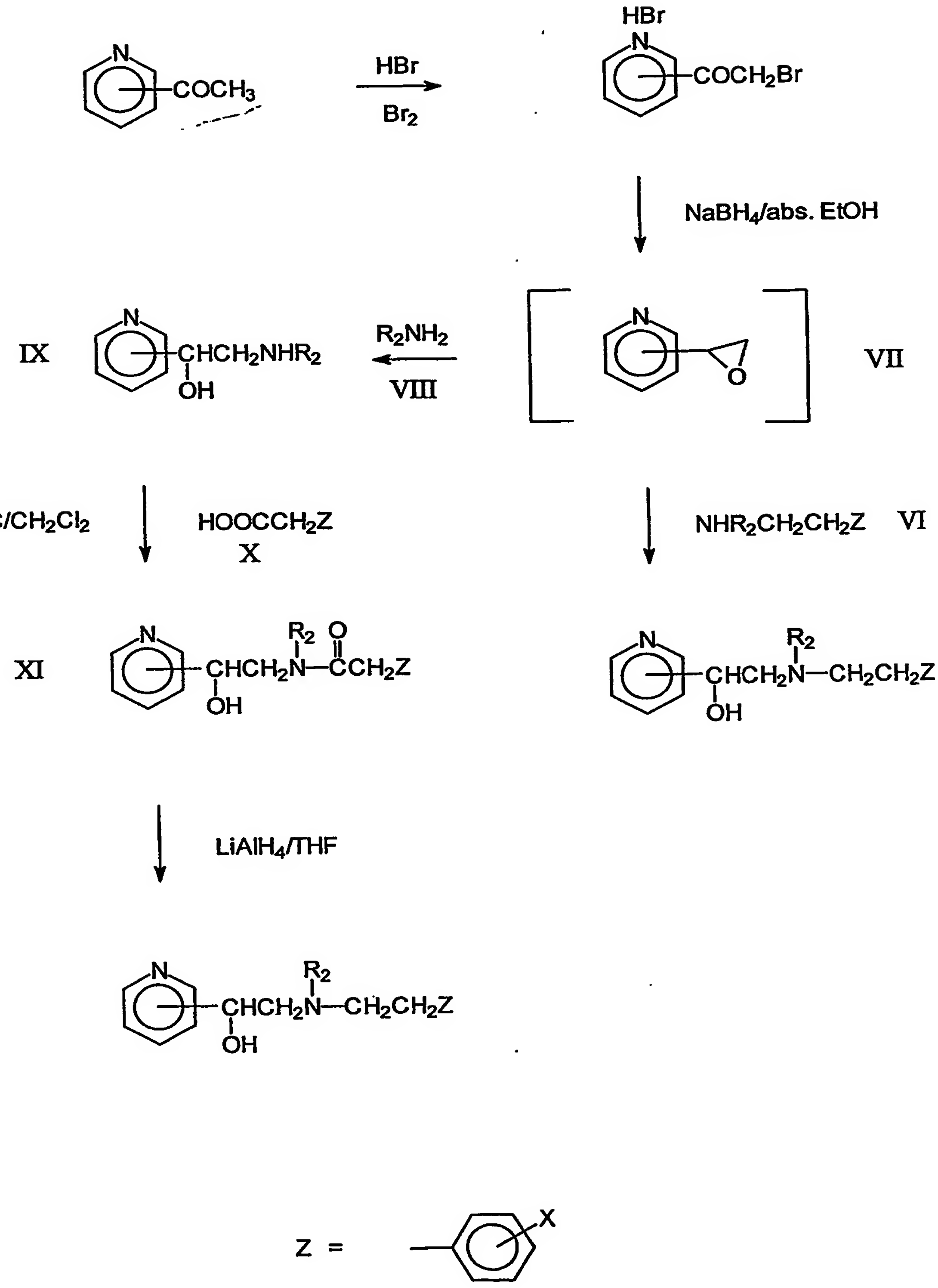

70
60
50
40
30
20
10
0
AU
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK38.2HBr
Negativna kontrola
Pravastatin
Lovastatin
Flukonazol

B


20
18
16
14
12
10
8
6
4
2
0
AU
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK-38.2HBr
normalni medij
pravastatin
lovastatin
flukonazol

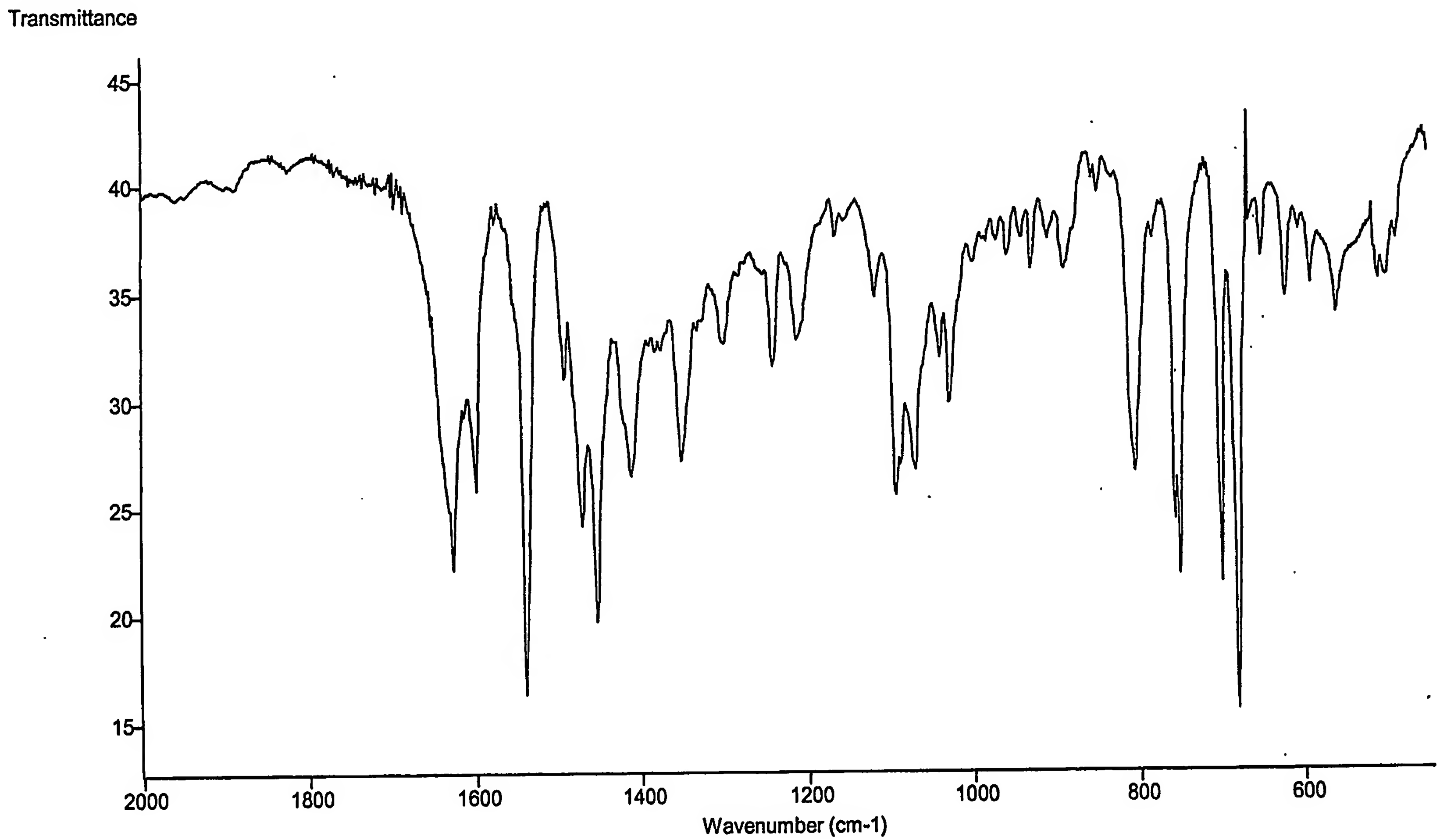
17.07.02

Slika 5



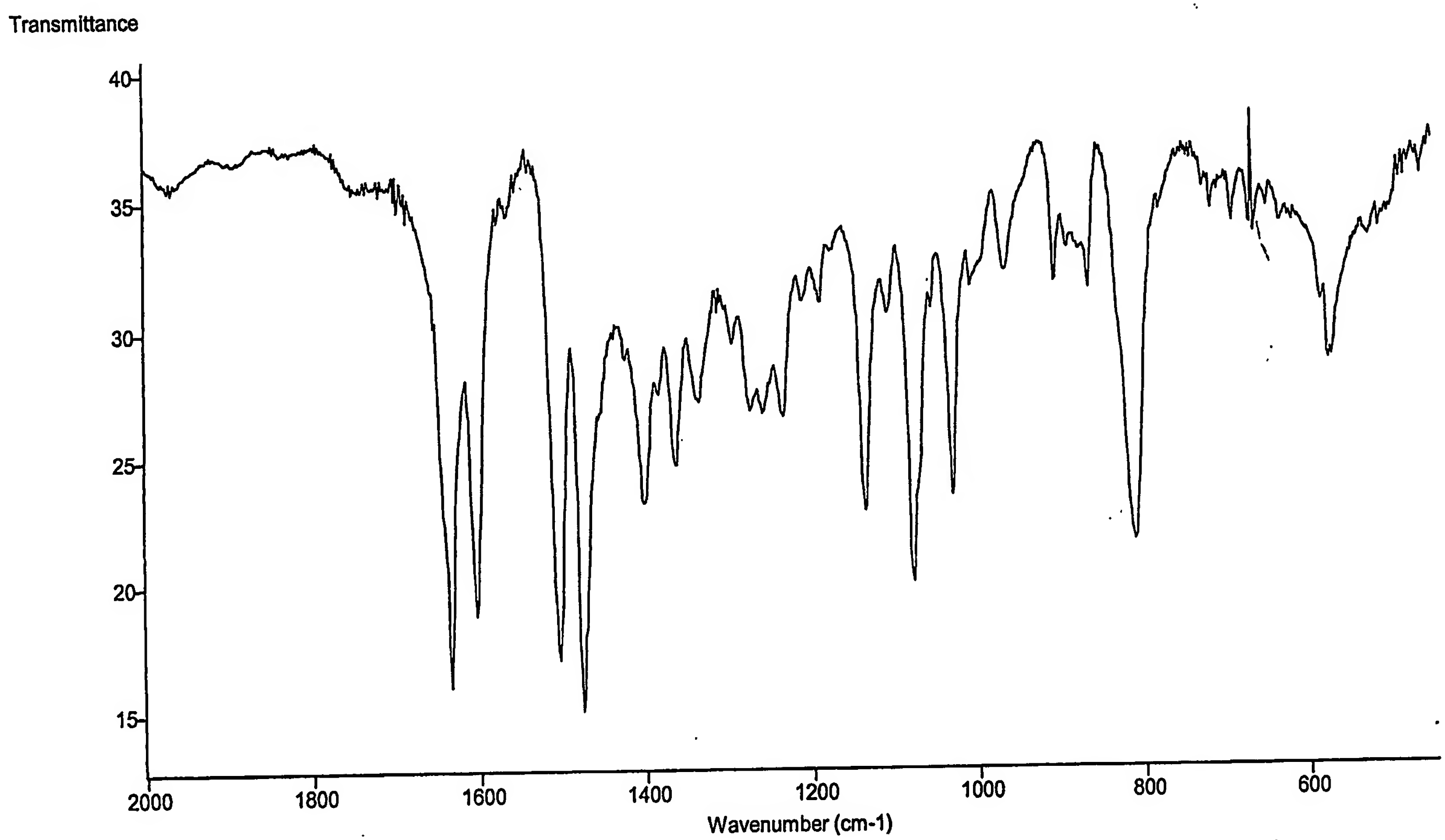
6/9

Slika 6


Transmittance
45
40
35
30
25
20
15
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

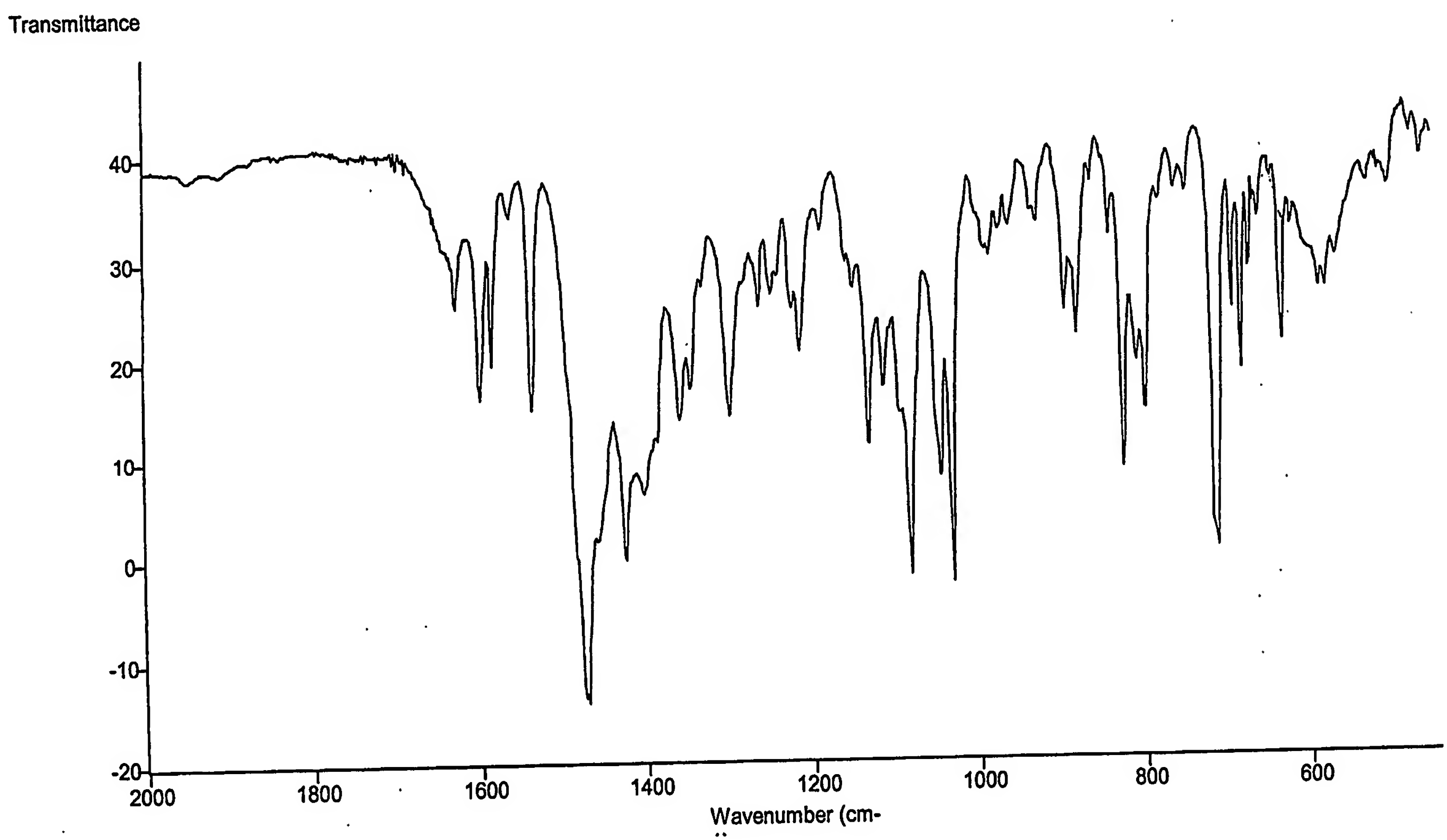
7/9

Slika 7

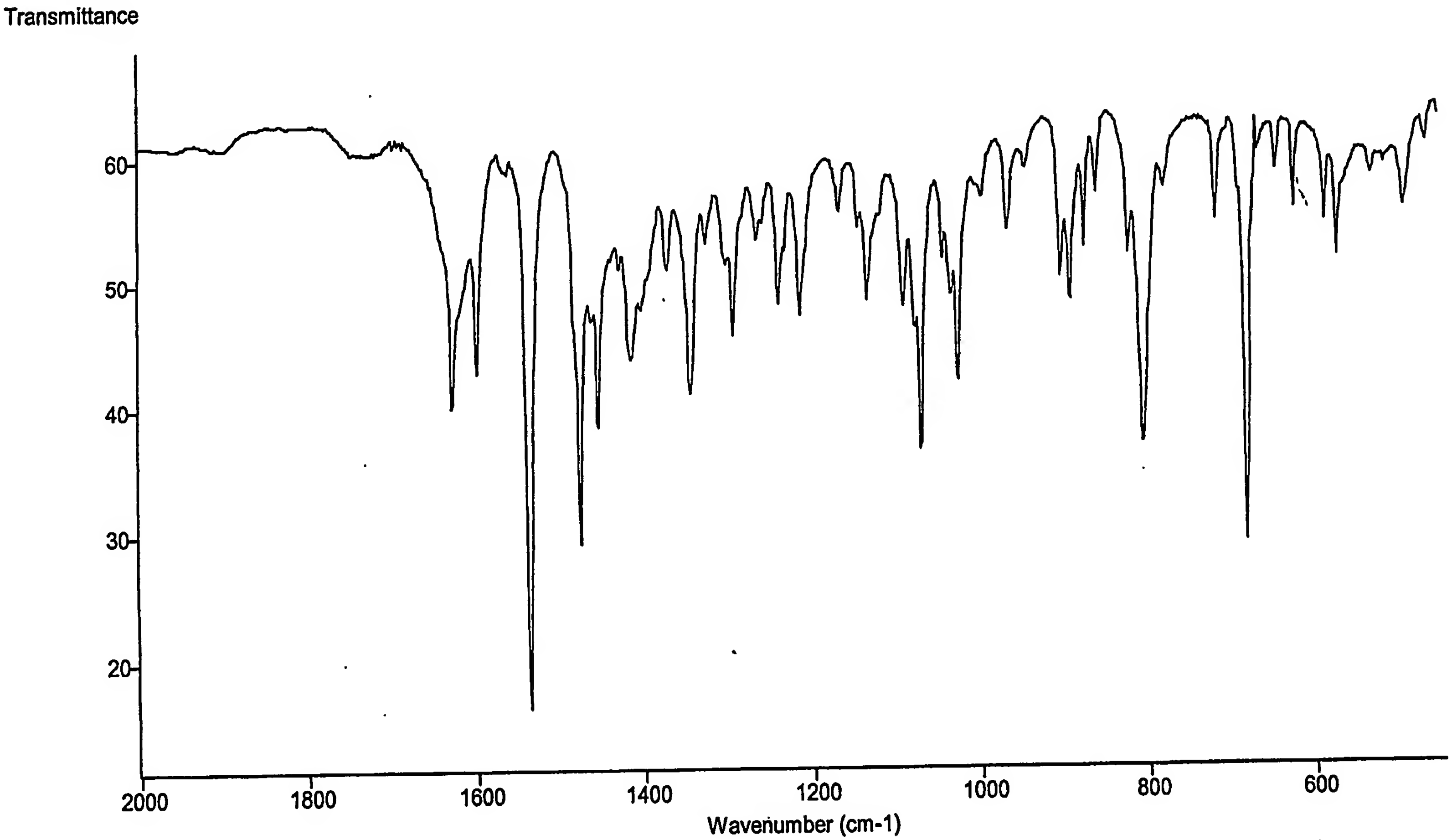


Transmittance
40
35
30
25
20
15
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

Slika 8


Transmittance
40
30
20
10
0
-10
-20
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-

Slika 9


Transmittance
60
50
40
30
20
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

REPUBLIC OF SLOVENIA

Ministry of Economic Affairs

SLOVENIAN INTELLECTUAL PROPERTY OFFICE

Certificate

Slovenian Intellectual Property Office hereby certifies that the document annexed hereto is a true copy of the patent application, as follows:

(22) Application Date:

17July 2002

(21) Application No.:

P-200200177

(54) Title:

Novel derivatives of pyridylethanol (phenylethyl) amines as inhibitors of cholesterol biosynthesis, processes for their preparation, and pharmaceutical compositions containing them

Ljubljana, 23 April 2003

Janez Kukec-Mezek
Government Counsellor

L.S.
Republic of Slovenia
Ministry of Economic Affairs
Slovenian Intellectual Property Office
Ljubljana

REPUBLIC OF SLOVENIA
MINISTRY OF SCIENCE AND TECHNOLOGY

SLOVENIAN INTELLECTUAL
PROPERTY OFFICE
1000 LJUBLJANA, Kotnikova 6

REQUEST FOR A PATENT GRANT

1. Address for correspondence: LEK d.d. Intellectual Property Verovškova 57, 1526 LJUBLJANA SLOVENIA Telephone: (01) 580 20 05 Fax: (01) 568 21 23 Code: LI / 797	Acknowledgement of the application *(for official use only)* Date of application receipt: 17 July 2002 Application number: **P** - 200200177 Stamp of the office and signature:
2. Applicant (Family name followed by given name and address; for a legal entity, full official designation LEK Pharmaceuticals d.d. Verovškova 57, 1526 Ljubljana Slovenia	

3. Representative: Registration No.:

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5. Title of invention:
NOVEL DERIVATIVES OF PYRIDYLETHANOL (PHENYLETHYL) AMINES AS INHIBITORS OF CHOLESTEROL BIOSYNTHESIS, PROCESSES FOR THEIR PREPARATION, AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

6. Claimed priority right:

7. Additional requests:
- ☐ application for a shortened duration patent
- ☐ preliminary publication after the expiry of ___months
- ☐ application is divided from the application no.:___

8. Statements:
- ☐ statement of common representative

REPUBLIC OF SLOVENIA
MINISTRY OF SCIENCE AND TECHNOLOGY

SLOVENIAN INTELLECTUAL
PROPERTY OFFICE
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9. Enclosures:

- x Description of the invention, having 18 pages 2x
- x Patent claim (claims), having 3 page; number of claims: 11 2x
- x Schemes (if required for patent description); number of sheets: 9 2x
- x Abstract
- x Voucher for the settlement of fees - to follow
- ☐ Declaration of depositing the biological material if it is an invention which cannot be described
- ☐ Authorisation to the representative
- ☐ General authorisation to the representative is deposited in the office under no. ....
- ☐ Declaration of priority right
- ☐ Information of additional applicants
- ☐ Information of additional inventors
- ☐ Presentation of nucleotide or amino acid sequence in the description
- ☐ Application was previously faxed or mailed in electronic form
- ☐ ____________________

Alenka Košak

____

Applicant's (representative's) family name
followed by given name and signature,

Novel derivatives of pyridylethanol (phenylethyl) amines as inhibitors of cholesterol biosynthesis, processes for their preparation, and pharmaceutical compositions containing them

Field of the invention

(Int. Cl. C 07 D 213/38, A 61 K 31/44)

The present invention belongs to the area of the active substances from the group of heterocyclic compounds, and the pharmaceutical industry and it relates to the novel derivatives of pyridylethanol (phenylethyl) amine, the processes for their preparation, pharmaceutical compositions containing them, and to their use for inhibiting cholesterol biosynthesis. The novel derivatives of pyridylethanol (phenylethyl) amine according to the invention are the receptors of sigma ligands, inhibitors of cholesterol biosynthesis at the level of sterol Δ7,8-isomerase and are suitable for the treatment of hypercholesterolemia and hyperlipemia in humans.

Technical problem

There is a constant need for new active substances that inhibit cholesterol biosynthesis, effective antihypercholestrolemic and antihyperlipemic agents which would provide a more targeted action in the therapy and with fewer side effects in comparison to the active substances known in the prior art.

Prior art

Because the high blood cholesterol level is a recognized risk factor in the onset of atherosclerosis, numerous investigations have been aimed at searching for a drug which would bring about reduced levels of blood cholesterol in the mammals and thus it would highly effective in the treatment of hypercholesterolemia and hyperlipemia. It has been established that lowering cholesterol biosynthesis by inhibitors of cholesterol biosynthesis is one of the modes of treatment.

Several inhibitors of cholesterol biosynthesis are known at the level of inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), as disclosed, for example, in US patent no. 4,231,938 (lovastatin), US patent no. 4,444,784 (simvastatin), US patent no. 4,346,227 (pravastatin sodium) or US patent no. 5,273,995 (atorvastatin) which are already used in the therapy and are the recognized commercial preparations Mevacor®, Sinvacor®, Lipitor®. These HMG-CoA reductase inhibitors, also known by the common name statins, significantly lower blood cholesterol levels.

Derivatives of pyridine-ethanolamine that are useful in the treatment of obesity and/or diabetes, especially in obese adult individuals, are known from US patent no. 4,800,206.

It is known that sigma ligands bind to sigma receptors that are structure homologues of sterol Δ8,7-isomerase (F. F. Moebius et al, Brit. J. Pharmacol.

(1997), 121, 1-6) and belong to the last portions of cholesterol biosynthesis. However, there are no active substances or drugs known in the current medicine that would inhibit cholesterol biosynthesis at the level of sterol Δ8,7-isomerase.

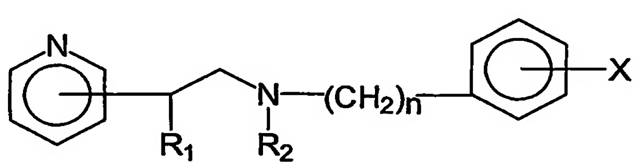
Problem solution description including examples

The aim of the present invention is to find new active substances that would significantly lower the level of blood cholesterol in the mammals by inhibiting cholesterol biosynthesis in the last portions of its biosynthesis pathway, that is, at the level of sterol Δ7,8-isomerase, thus, have a more selective inhibitory action than the action of known statins which inhibit HMG-CoA reductase in the early portion of cholesterol biosynthesis pathway.

The use of the novel compounds of this invention would permit a more targeted therapeutic action with fewer side effects in comparison with the active substances already approved in the therapy.

This problem has been solved by the present invention which relates to novel pyridylethanol (phenylethyl) amine derivatives, to the processes for their preparation, to the pharmaceutical compositions containing them and the use of the compounds in accordance with the invention for the treatment of hypercholesterolemia and hyperlipemia.

New pyridylethanol (phenylethyl) amines of this invention are compounds of general formula I



I

wherein

n is an integer from 1 to 4

R_1 is a hydrogen atom, hydroxyl group or lower C_{1-6} alkoxy group

R_2 is a hydrogen atom or a straight or branched lower C_{1-6} alkyl group

X is hydrogen, fluorine, chlorine, bromine, hydroxyl group, trifluoromethyl group, 3,4-di-Cl, 2,4-di-Cl or lower C_{1-6} alkoxy group

as well the physiologically acceptable acid addition salts thereof.

The term lower alkyl group denotes straight- or branched-chain lower alkyl group with 1 to 6, preferably 1 to 4, carbon atoms (C_{1-6} alkyl) such as methyl, ethyl, n-propyl, isopropyl, n-butyl and isobutyl group. The term lower alkoxy group denotes alkoxy group with 1 to 6, preferably 1 to 4, carbon atoms (C_{1-6} alkoxy) such as methoxy, ethoxy, propoxy, isopropoxy, butoxy and isobutoxy group.

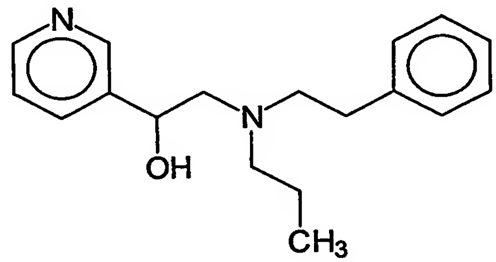
The compounds of formula I form salts with acids and these salts are also the part of the invention. Examples of such salts are the salts with physiologically compatible mineral acids such as, for example, hydrochloric acid, hydrobromic acid, phosphoric acid; or with organic acids such as, for example, methanesulfonic acid, citric acid, oxalic acid, maleic acid, benzenesulfonic acid and others.

New compounds of this invention contain at least one asymmetric carbon atom and can, therefore, exist as optically active enantiomers, as diastereomers or as racemates.

The compounds of formula I in which n = 2 and in which R_1 is a hydroxyl group, R_2 is a methyl or n-propyl group and X is a hydrogen atom or two atoms of chlorine in the positions 3 and 4 of the phenyl nucleus, are the novel derivatives of pyridylethanol (phenylethyl) amine and are the preferred compounds in accordance with the invention.

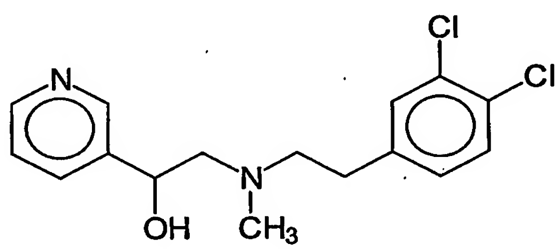
Of the compounds, mentioned above, preferred compound are:

1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino)ethanol and a dihydrobromide salt of formula II thereof (signature BK-31 in descriptions and figures)



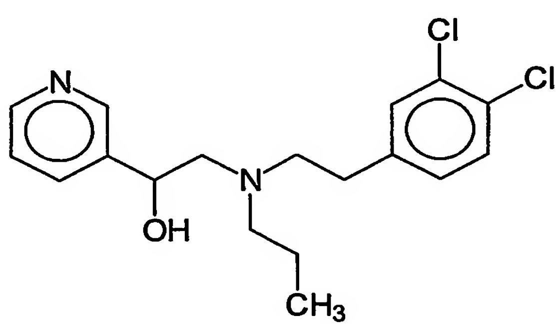
II

1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-methylamino)ethanol and a dihydrobromide salt of formula III thereof (signature BK-33 in descriptions and figures)



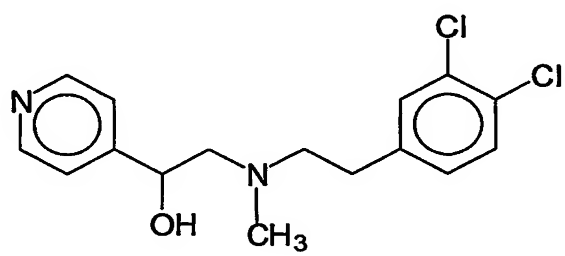
III

1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-propylamino)ethanol and a dihydrobromide salt of formula IV thereof (signature BK-35 in descriptions and figures)



IV

and 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-methylamino)ethanol and a dihydrobromide salt of formula V thereof (signature BK-38 in descriptions and figures)



V

Of the above mentioned compounds of this invention especially preferred compound is 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-propylamino)ethanol and a dihydrobromide salt (BK-35.2HBr) thereof as an inhibitor of cholesterol biosynthesis and thus appropriate for the treatment of hypercholesterolemia and hyperlipemia.

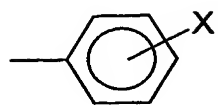
The compounds of this invention may be prepared in two different ways which are shown in the following scheme as variant (a) and variant (b):

variant a):

alkylating secondary amines of formula VI

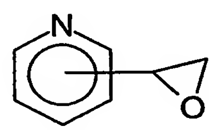
$$NHR_2CH_2CH_2Z \qquad VI$$

wherein R_2 is as defined above and Z is a group



wherein X is as defined above,

with pyridyloxirane (pyridyl ethylene oxide) of formula VII



VII

to the desired title pyridylethanol (phenylethyl) amines of formula I and, if desired, converting them into to the physiologically acceptable acid addition salts thereof.

Secondary amines of formula VI may be prepared by alkylating primary amines of formula XII

$H_2N - CH_2CH_2Z$ XII

with alkyl iodides of formula XIII

R_2J XIII

according to the following reaction scheme:

$$H_2N - CH_2CH_2Z + R_2J \longrightarrow HNR_2 - CH_2CH_2Z$$

wherein substituents R_2 and Z are as defined above.

Primary amines of formula XII and alkyl iodides of formula XIII are known and commercially available chemicals.

At 2-, 3- or 4- substituted pyridyloxirane of formula VII in the process of alkylating secondary amines of formula VI is prepared *in situ* by transformation at 2-, 3- or 4-substituted bromo-acetylpyrine hydrobromide with complexed metal hydrides, such as sodium boronhydride in an inert solvent such as lower aliphatic alkanol, for example, ethanol at a temperature about room temperature.

At 2-, 3- or 4-substituted bromo-acetylpyridine hydrobromide is prepared by transformation of the original at 2-, 3- or 4-substituted acetylpyridine which are known and commercially available chemicals for bromination with bromine and hydrobromic acid.

The alkylation step of secondary amines of formula VI with pyridyloxirane of formula VII is carried out at a temperature of about room temperature to reflux temperature of the reaction mixture, in an inert solvent such as lower aliphatic alkanol, for example, ethanol. The crude pyridylethanol (phenylethyl) amines of formula I formed are isolated and purified by common procedures known in the prior art, preferably by column chromatography.

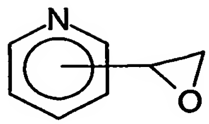
Variant (b):

Alkylating primary amines of formula VIII

$$R_2NH_2$$

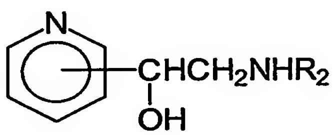
wherein R_2 is as defined above,

with pyridyloxirane of formula VII



VII

to intermediate compounds of formula IX



IX

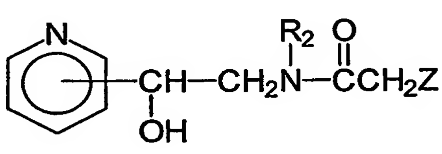
wherein R_2 is as defined above,

and condensing with the derivatives of phenyl acetic acid of formula X

$$HOOCCH_2Z \qquad X$$

wherein Z is as defined above,

to new intermediate compounds of formula XI



XI

wherein substituents R_2 and Z are as defined above,

and reducing them to the desired title pyridylethanol (phenylethyl) amines of formula I, and, if desired, converting them into the physiologically acceptable acid addition salts thereof.

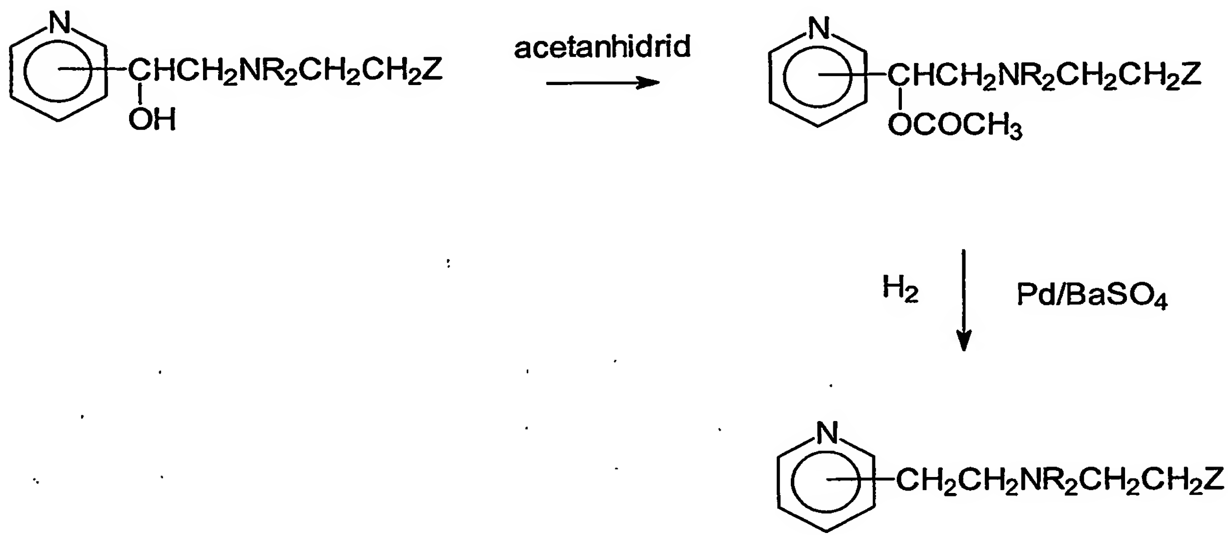
Primary aliphatic amines of formula VIII, such as methylamine or n-propylamine, are known and commercially available chemicals which are alkylated with pyridyloxirane of formula VII in an inert solvent, such as lower aliphatic alkanol, for example ethanol, to intermediate compounds of formula IX. These intermediate compounds are condensed with the derivatives of phenyl acetic acid of formula X wherein a substituent Z is as defined above, in an inert solvent and at a temperature about room temperature. Condensing agents known in the art may be used as a condensing agent, such as dicyclohexylcarbodiimide (DCC), as an inert solvent, for example, methylene chloride (dichloromethane).

In the final step of the synthesis, a carbonyl group in the novel intermediary compounds XI is reduced to an alcohol group. The reaction is carried out with conventional reducing agents, preferably with those suitable for reduction of the carbonyl group to the group $-R_2HN-CO-$. Especially suitable is a complex metal hydride, such as $LiAlH_4$ in an inert solvent, preferably in ether, such as tetrahydrofuran (THF), diethyl ether, dioxane and similar. The desired title pyridylethanol (phenylethyl) amines of formula I formed are isolated and purified in a conventional manner, preferably by column chromatography on silica gel and then, if desired, they are converted into the physiologically acceptable acid addition salts thereof.

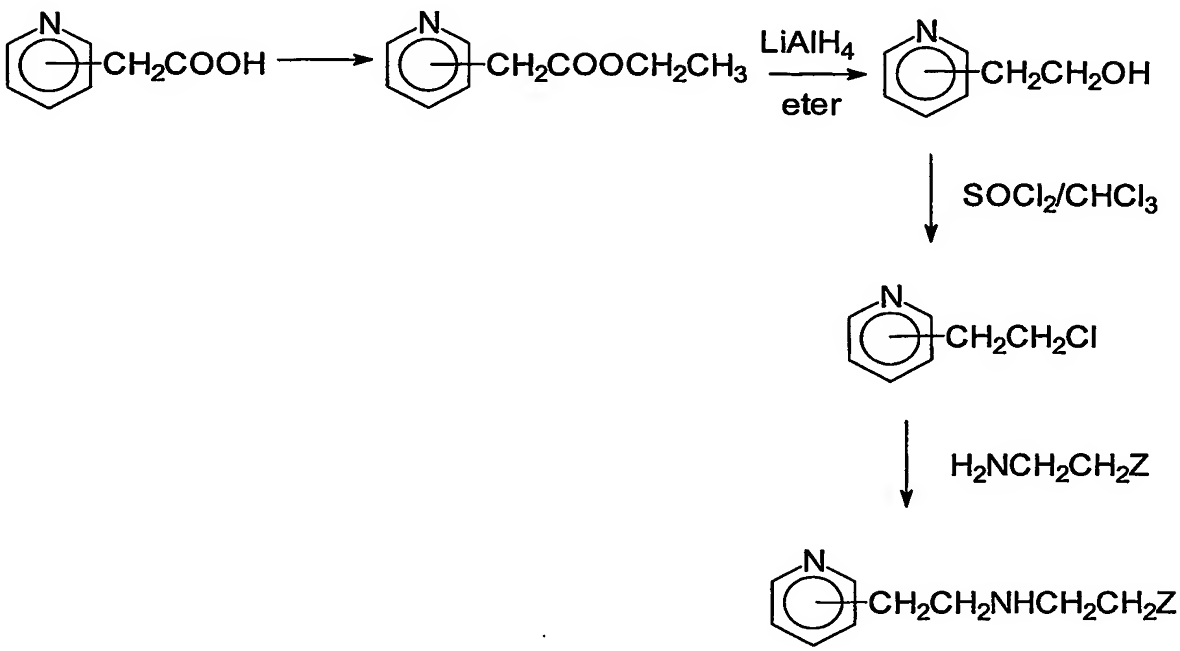
The processes for preparation of the novel derivatives of pyridylethanol (phenylethyl) amine of formula I in accordance with the variants (a) and (b) are shown in figure 5.

The synthesis of the novel derivatives of pyridylethanol(phenylethyl) amines of formula I in which R_1 is a hydrogen atom may be performed so that the novel compounds of formula I in accordance with the invention wherein R_1 is a hydroxyl

group, are first acetylated in a conventional manner, for example, with acetanehydride and then the O-acetyl compound formed is catalytically hydrogenated by common methods, such as, with palladium on a carrier, for example, barium sulfate, according to the following variant c)



By the other variant the novel derivatives of pyridylethanol (phenylethyl) amine of formula I may be prepared wherein R1 represents a hydrogen atom, according to the following variant d)



The original at 2-, 3- or 4-substituted pyridyl acetic acid is esterified in a conventional manner known in the prior art, for example, by transforming it to ethyl ester of pyridylacetic acid thereof which is then reduced with conventional reductants, preferably with those for reduction of the ester group to an alcohol group. Particularly suitable is a complexed metal hydride, such as lithium aluminum hydride ($LiAlH_4$) in an inert solvent, preferably in ether, such as diethyl ether, tetrahydrofuran, dioxane and the like. By this procedure produced 2, 3 or 4-substituted pyridyl ethanol is transformed to 2-, 3- or 4-substituted pyridyl ethylenechloride with common chlorinating agents, such as thionyl chloride in an inert solvent, such as chloroform. The produced substituted pyridylethylene chloride is used to alkylate primary amines of formula VI to produce the title derivatives of pyridylethanol (phenylethyl) amines of formula I wherein R_1 represents a hydrogen atom.

In accordance with the invention goal, the effect of the novel derivatives of pyridylethanol (phenylethyl) amine as ligands of sigma receptors on inhibition of cholesterol biosynthesis was assessed. An *ex vivo* method of metabolic labeling of immortal human hepatocytes was employed. The radioactively labelled early precursor of cholesterol [^{3}H] acetate was added to cells with or without addition of

sigma ligands. Two independent experiments of metabolic labeling and sterol analysis were performed. The results of both analyses are reproducible and show that the tested substances significantly lower cholesterol synthesis.

Of novel ligands of sigma receptors of this invention, the highest potential to inhibit cholesterol biosynthesis is exhibited by the substance 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-propylamino)ethanol, in the form of dihydrobromide salt (signature BK 35 . 2HBr).

Recently, it has been established that sigma ligands bind to sigma receptors that are structure homologues of sterol Δ8,7-isomerase since they belong to the same gene family. Sterol Δ8,7-isomerase contributes to the late portion of cholesterol biosynthesis, as evident from figure 1. Figure 1 shows that the most commonly used substrates are Δ8-cholestenol and zymosterol which differ in the saturation of the side chain at position Δ24,25. Figure 2 represents cholesterol biosynthesis with marked sites of action of inhibitors of cholesterol biosynthesis.

The effect of novel pyridylethanol (phenylethyl) amines as sigma ligands in accordance with the invention is more selective than the effect of statins, used in the therapy, such as lovastatin or pravastatin, which inhibit HMG-CoA reductase that belongs to the early portion of cholesterol biosynthesis.

With novel pyridylethanol (phenylethyl) amines of this invention a more selective action with fewer side effects is provided due to the inhibition of cholesterol biosynthesis in late steps of this biosynthesis pathway. Consequently, these substances are particularly useful for the treatment of hypercholesterolemia and hyperlipemia. These effects of the novel pyridinylethanol (phenylethyl) amines were truly unexpected as insofar in medical practice and therapy lack of substances that would lower cholesterol level by targeting enzymes in late steps of cholesterol biosynthesis.

Application of the novel pyridylethanol (phenylethyl) amines of formula I of this invention markedly decreases the pathologically increased blood cholesterol levels in treated patients. The dosage and frequency of application depend on the characteristics of an individual drug, its bioavailability and pharmacokinetic characteristics, and the patient's condition.

Pharmaceutical preparations contain the active substance together with the physiologically compatible organic or inorganic support, such as water, lactose, starch and its derivatives, magnesium stearate, talc, plant oils and similar. Pharmaceutical preparations are preferably administered orally, such as in the form of tablets, capsules, pills, powders, granulates, solutions, syrups, suspensions, elixirs and similar. Administration can be also carried out parenterally, for example, in the form of sterile solutions, suspensions or emulsions. Pharmaceutical preparations can be sterilized and/or include ingredients, such as, preservatives, stabilizers, emulsifiers, buffering substances and other additives.

The present invention is illustrated but in no way limited by the following examples:

EXAMPLE 1

1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino)ethanol (BK 31)

Preparation of the starting compounds:

N-propyl-(β-phenylethyl)amine

1.1 ml (9.5 mmol) of phenylethylamine, 0.8 ml (9.5 mmol) of n-propyl-iodide, 5 ml of triethylamine and 5 ml of THF (tetrahydrofuran) were placed in a flask, and the reaction mixture was heated at reflux temperature of the reaction mixture for 3.5 hours and then cooled. A salt formed was filtered off, the solution was evaporated and a desired compound was purified by column chromatography on silica gel

(silica gel 60, mobile phase: $CHCl_3 : CH_3OH = 10 : 3$). This yields 0.62 g (40%) of N-propyl-(β-phenylethyl)amine in the form of the oil (molecular weight: 163.264, formula: $C_{11}H_{17}N$).

3-bromoacetylpyridine hydrobromide

To 10 g (82.5 mmol) of 3-acetylpyridine was added 30 ml of 48% hydrobromic acid. The reaction mixture was heated to 70°C, and 4.2 ml of bromine was added dropwise with stirring. After completed addition of bromine, the reaction mixture was stirred further for 15 minutes at the same temperature and cooled on ice. A crystalline compound formed was filtered off and thoroughly washed with acetone. This yields 21 g (90%) of 3-bromoacetylpyridine hydrobromide, melting point 195-200°C.

Preparation of the title 1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino)ethanol

To 1.01 g (3.6 mmol) of 3-bromoacetylpyridine hydrobromide was added 20 ml of absolute ethanol and 0.5 g (13.2 mmol) of sodium boronhydride. The reaction mixture was stirred at 20°C for 2 hours, filtered and to the filtrate containing 3-pyridyloxirane was added 0.96 g (5.9 mmol) of N-propyl-(β-phenylethyl)amine. The reaction mixture was heated at reflux temperature of the reaction mixture for 4 hours and evaporated to a dry residue, and to it was added 20 ml of chloroform. A solid portion was filtered off, the filtrate was evaporated, and an oil residue formed was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3 : CH_3OH = 10 : 3$). This yields 0.56 g (55%) of the title compound in the form of the oil base.

0.56 g (2 mmol) of a purified oil base of 1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino) ethanol was dissolved in 5 ml of acetone. The resulting solution was cooled on ice, and with stirring 2.5 ml of ethanolic solution of hydrobromic acid solution (0.35 g (4.3 mmol HBr)) was added. To a precipitate formed was added 3 ml of diethyl ether. After stirring for 2 hours on ice, a crystalline product was

filtered off and washed with diethyl ether. This yields 0.7 g (80%) of 1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino)ethanol dihydrobromide, melting point 113–120°C (molecular weight: 446.238, gross formula: $C_{18}H_{24}N_2O$. 2HBr).

^1H NMR spectrum, D_2O, ppm according to DSS (0ppm): 8.89, 8.80 (2H), 8.65, 8.57 (1H), 8.10 (1H), 7.38 (5H), 5.47 (1H), 3.7-3.1 (8H), 1.80 (2H), 0.97 (3H).

IR (infra-red) spectrum (KBr disc) is shown in figure 6.

EXAMPLE 2

1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-metylamino)ethanol (BK 33)

Preparation of 1-(3-pyridyl)-2-metylaminoethanol

To 1.01 g (3.6 mmol) of 3-bromoacetylpyridine hydrobromide, prepared as described in Example 1, was added 20 ml of absolute ethanol and 0.5 g (13.2 mmol) of sodium boronhydride, a reaction mixture was stirred at 20°C for 2 hours and filtered. To a filtrate containing 3-pyridyloxirane was added 1.3 ml of 33% ethanolic solution of methylamine and heated at reflux temperature of the reaction mixture for 5 hours. The reaction mixture was then evaporated to a dry residue and to it was added 20 ml of chloroform. A solid portion was filtered off, the filtrate was evaporated and an oil residue formed was purified by column chromatography on silica gel (mobile phase: $CHCl_3 : CH_3OH = 10 : 3$). This yields 0.33 g (60%) of a title compound in the form of the oil base (molecular weight: 152.196, gross formula: $C_8H_{12}N_2O$).

Preparation of 1-(3-pyridyl)-2-(N-(2-(3,4-diclophenyl)acetyl-N-metylamino)ethanol

To a flask containing 542 mg (2.6 mmol) of DCC (dicyclohexylcarbodiimide) was added 2 ml of methylene chloride, and a solution of 538 mg (2.6 mmol) of 3,4-dichlorophenyl acetic acid in 3 ml of methylene chloride was added dropwise with

stirring resulting in the formation of a precipitate. After stirring for 5 minutes, 400 mg (2.6 mmol) of 1-(3-pyridyl)-2-methylaminoethanol was added to the reaction mixture and stirred further for 1 hour at 20°C. A precipitate formed was filtered off and the solution was evaporated. The evaporated filtrate was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: CH_3OH = 10 : 0.5). This yields 715 mg (80%) of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl-N-methylamino)ethanol (molecular weight: 339.224, gross formula: $C_{16}H_{16}N_2O_2Cl_2$)

Preparation of the title 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl-N-methylamino)ethanol (BK-33)

0.53 g (13.9 mmol) of lithium aluminum hydride ($LiAlH_4$) was placed into a flask, 6 ml of anhydrous tetrahydrofuran (THF) was added and a mixture was cooled on ice. To the reaction mixture was added dropwise with stirring a solution of 1.1 g (3.2 mmol) of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-ethylamino) ethanol in 10 ml of anhydrous tetrahydrofuran (THF). After completed addition, the reaction mixture was stirred further for 1 hour at 20°C, cooled on ice and with vigorous stirring was added stepwise 6.5 ml of 15% NaOH and then 16 ml of methylene chloride (CH_2Cl_2). An organic phase was separated, dried on anhydrous Na_2SO_4 and evaporated on a rotavapor resulting in the formation of an oil residue which was then purified by column chromatography on silica gel (silica gel 60, mobile phase: CH_3OH : ethyl acetate = 10 : 2). This yields 0.63 g (60%) of a title compound in the form of the oil base.

0.60 g (1.84 mmol) of a purified oil base was dissolved in 3.5 ml of acetone. The solution was cooled on ice and with stirring was added 2.4 ml of ethanolic solution of hydrobromic acid (0.328 g HBr; 4.1 mmol). To a residue formed was added 2 ml of diethyl ether. After stirring the reaction mixture for 2 hours on ice, a crystalline product formed was filtered off and washed with diethyl ether. This yields 0.76 g (80%) of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-methylamino)ethanol dihydrobromide, melting point 157–161°C (molecular weight: 487.074, gross formula: $C_{17}H_{18}N_2OCl_2$. 2HBr).

^{1}H NMR spectrum; D_2O, ppm according to DSS (0ppm): 8.90 (1H), 8.78 (1H), 8.64 (1H), 8.10 (1H), 7.50 (2H), 7.24 (1H), 5.50 (1H), 3.52 (4H), 3.08 (5H).

IR spectrum (KBr disc) is shown in figure 9.

EXAMPLE 3

1-(3-pyridyl)-2-(N-(2-(3,4-diclorophenyl)ethyl)-N-propylamino)ethanol (BK-35)

Preparation of 1-(3-pyridyl)-2-propylaminoethanol

To 1.01 g (3.6 mmol) of 3-bromoacetylpyridine hydrobromide, prepared as described in Example 1, was added 20 ml of absolute ethanol and 0.5 g (13.2 mmol) of sodium boronhydride ($NaBH_4$). The reaction mixture was stirred at 20°C for 2 hours and filtered. To the filtrate containing 3-pyridyloxirane was added 0.7 ml of n-propylamine and heated at reflux temperature of the reaction mixture for 5 hours. The reaction mixture was then evaporated to a dry residue and to it was added 20 ml of chloroform, a solid portion was filtered off, the filtrate was evaporated and an oil residue formed was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: ethyl acetate = 10 : 2). This yields 0.33 g (50%) of title compound in the form of the oil base (molecular weight: 367.278, gross formula: $C_{18}H_{20}N_2O_2Cl_2$)

Preparation of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-propylamino)ethanol

To a flask containing 630 mg (3.1 mmol) of DCC (dicyclohexylcarbodiimide) was added 3 ml of methylene chloride and with stirring a solution of 625 mg (3.1 mmol) of 3,4-dichlorophenyl acetic acid in 5 ml of methylene chloride was added dropwise resulting in the formation of a precipitate. The reaction mixture was stirred for 5 minutes, and to it was added 550 mg of 1-(3-pyridyl)-2-

methylaminoethanol in 6 ml of methylene chloride, it was stirred further for 1 hour at 20°C. A precipitate formed was filtered off, and the resulting solution was evaporated. The evaporated filtrate was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: CH_3OH = 10 : 0.5). This yields 0.56 g (50%) of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-propylamino)ethanol in the form the oil (molecular weight: 367.278, gross formula: $C_{18}H_{20}N_2O_2Cl_2$).

Preparation of the title 1-(3-pyridyl)-2-[N-(2-(3,4-dichlorophenyl)ethyl)-N-propylamino]ethanol (BK-35)

0.43 g (11.4 mmol) of lithium aluminum hydride ($LiAlH_4$) was placed into a flask, 6 ml of anhydrous tetrahydrofuran (THF) was added and a mixture was cooled on ice. To the reaction mixture was added dropwise with stirring a solution of 1 g of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-propylamino)ethanol in 10 ml of anhydrous THF. After completed addition, the reaction mixture was further stirred for 1 hour at 20°C, cooled on ice and with vigorous stirring 6.4 ml of 15% NaOH was added stepwise and then 16 ml of methylene chloride. An organic phase was separated, dried on anhydrous Na_2SO_4 and evaporated on a rotavapor. The evaporated residue was purified by column chromatography on silica gel (silica gel 60, first mobile phase: $CHCl_3$: CH_3OH = 10 : 0.5; second mobile phase: ethyl acetate: CH_3OH = 10 : 1.5). This yields 0.58 g (60%) of a title compound in the form of oil base.

0.50 g (1.4 mmol) of an obtained purified oil base was dissolved in 4 ml of acetone. The resulting solution was cooled on ice and with stirring was added 1.1 ml of ethanolic solution of hydrobromic acid (0.25 g HBr; 3.1 mmol). A white precipitate was formed and to it was added 3 ml of diethyl ether and after stirring on ice for 2 hours, a crystalline product formed was filtered off and washed with diethyl ether. This yields 0.62 g (85%) of 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-propylamino)ethanol dihydrobromide, melting point 136–139°C (molecular weight: 515.124; gross formula: $C_{18}H_{22}N_2OCl_2 . 2HBr$)

^1H NMR spectrum, D_2O, ppm according to DSS (0ppm): 8.56 (2H), 8.01 (1H), 7.59 (1H), 7.50 (2H), 7.23 (1H), 5.25 (1H), 3.50 (4H), 3.30 (2H), 3.11 (2H), 1.78 (2H), 0.96 (3H)

IR spectrum (KBr disc) is shown in figure 8.

EXAMPLE 4

1-(4-pyridyl)-2(N-(2-(3,4-dichlorophenyl)ethyl)-N-methylamino)ethanol (BK-38)

Preparation of 1-(4-pyridyl)-2-methylaminoethanol

To 1.01 g (3.6 mmol) of 4-bromoacetylpyridine hydrobromide, prepared as described in Example 1, was added 20 ml of absolute ethanol and 0.5 g (13.2 mmol) of sodium boronhydride, and the reaction mixture was stirred at 20°C for 2 hours, then filtered and to the filtrate containing 4-pyridyloxirane was added 1.3 ml 33% ethanolic solution of methylamine. The reaction mixture was heated at reflux temperature of the reaction mixture for 3 hours, evaporated to a dry residue and to it was added 20 ml of chloroform, and a solid portion was filtered off. The filtrate was evaporated and an obtained oil residue was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: ethyl acetate = 10 : 2). This yields 0.30 g (55%) of a title compound in the form of the oil base (molecular weight: 152.196, gross formula: $C_8H_{12}N_2O$).

Preparation of 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-methylamino)ethanol

To a flask containing 0.54 g (2.6 mmol) of DCC (dicyclohexylcarbodiimide) was added 2 ml of methylene chloride and 0.54 g (2.6 mmol) of 3,4-dichlorophenyl acetic acid in 4 ml of methylene chloride was added dropwise to produce the precipitate. The reaction mixture was stirred for 5 minutes and 400 mg (2.6 mmol) of 1-(4-pyridyl)-2-methylaminoethnol in 3 ml of methylene chloride was added and

was stirred further for 1 hour at 20°C. A precipitate formed was filtered off, and the resulting solution was evaporated. The evaporated filtrate was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: CH_3OH = 10 : 0.5). This yields 0.53 g of 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-methylamino)ethanol.

Preparation of the title 1-(4-pyridyl)-2-[N-(2-(3,4-dichlorophenyl)ethy)-N-methylamino]ethanol (BK-38)

510 mg (13.5 mmol) of lithium aluminum hydride ($LiAlH_4$) was placed into a flask, 6 ml of anhydrous THF was added and a mixture was cooled on ice. To the reaction mixture was added dropwise with stirring a solution of 1.02 g (3 mmol) of 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)acetyl)-N-methylamino)ethanol in 10 ml of anhydrous THF. After completed addition, the reaction mixture was further stirred for 1 hour at room temperature, cooled on ice and with vigorous stirring 6.6 ml of 15% NaOH was added stepwise and then 16 ml of methylene chloride. An organic phase was separated, dried on anhydrous Na_2SO_4 and evaporated on a rotavapor to an oil residue which was purified by column chromatography on silica gel (silica gel 60, mobile phase: $CHCl_3$: CH_3OH = 10 : 1. This yields 0.67 g (85%) of 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-methylamino)ethanol dihydrobromide, melting point 191 - 194°C (molecular weight: 484.074; gross formula: $C_{17}H_{18}N_2OCl_2$. 2 HBr).

^1H NMR spectrum, D_2O, ppm according to DSS (0ppm): 8.81 (2H), 8.14 (2H), 7.47 (2H), 7.22 (1H), 5.54 (1H), 3.50 (4H), 3.08 (5H)

IR spectrum (KBr disc) is shown in figure 7.

EXAMPLE 5

Testing of four sigma receptors, (BK-31 . 2HBr, BK-33 . 2HBr, BK-35 . 2HBr and BK-38 . 2HBr) from examples 1 to 4, inhibitors of cholesterol biosynthesis as the level of sterol Δ7,8-isomerase

The inhibitory effect on cholesterol biosynthesis of our novel ligands of sigma receptors BK-31 . 2 HBr, BK-33 . 2HBr, BK-35 . 2HBr and BK-38 . 2HBr), prepared according to examples 1 to 4, was evaluated. An *ex vivo* method of metabolic labeling of immortal human hepatocytes was applied. The radiolabel led precursor of cholesterol biosynthesis [^{3}H] acetate was added to the cells with or without the addition of sigma ligands. Finally, two independent experiments of metabolic labeling and sterol analysis were performed for each compound.

Materials and methods

Cell culture and addition of sigma receptors

The immortal human hepatocyte cell line $HepG_2$ was split to the 75 cm^2 flasks in the ratio 1:2, two flasks for each condition. The cells were cultured in the DMEM culture (L-arginine.HCl 0.084 g/l, L-cysteine.2HCl 0.0626 g/l, L-glutamine 0.584 g/l, glycine 0.03 g/l, L-histidine.HCl.H_2O 0.042 g/l, L-isoleucine 0.105 g/l, L-leucine 0.105 g/l, L-lysine.HCl 0.146 g/l, L-methionine 0.03 g/l, L-phenylalanine 0.066 g/l, L-serine 0.042 g/l, L-threonine 0.095 g/l, L-thryptophan 0.016 g/l, L-tyrosine 2Na.2H_2O 0.10379 g/l, L-valine 0.094 g/l, choline chloride 0.004 g/l, folic acid 0.004 g/l, myo-inositol 0.0072 g/l, niacinamide 0.004 g/l, D-pantothenic acid 0.004 g/l, pyridoxal.HCl 0.004 g/l, riboflavin 0.0004 g/l, thiamine.HCl 0.004 g/l, calcium chloride.2H_2O 0.265 g/l, ferric nitrate . 9 H_2O 0.0001 g/l, magnesium sulfate [anhydride] 0.09767 g/l, potassium chloride 0.4 g/l, sodium chloride 6.4 g/l, monobasic sodium phosphate [anhydride] 0.109 g/l, glucose 4.5 g/l and phenol red, Na 0.0159 g/l) with 5% bovine serum and 1% L-glutamine. After 24 hours a medium with 100 μM ligands of sigma receptors (BK-31 . 2HBr, BK-33 . 2HBr, BK-

35 . 2HBr and BK-38 . 2HBr) was added to the cells. The known inhibitors of cholesterol biosynthesis, 100 μM lovastatin or pravastatin, both the inhibitors of HMG-CoA reductase, and 100 μM fluconazole that inhibits enzymes of the P450 family to which lanosterol 14α - demetylase (CYP51) also belongs, were used as positive controls. The cells grown in normal media without the addition of inhibitors served as negative control. The medium was exchanged after 24 hours. After 48 hours, 40 μCi [^{3}H] acetate was added per 1 ml of the medium (400 μCi per flask). The medium was aspirated after 24 hours, and the cells were tryptinized with 2 ml of trypsine. The cells were collected in 4 ml of the medium, centrifuged, and the cell pellet resuspended in distilled water (1 ml per flask). The cells were homogenized by freeze-thawing. Sterols were extracted from the homogenate. Protein concentration was determined in the homogenate with the Bio-rad reagent according to the recommended protocol of the producer.

Sterol extraction

The homogenate was transferred into glass vials with an inert cover. 3 ml of the extraction solution (75% n-heptane : 25% isopropanol (vol/vol)) was added. The closed vials were shaken vigorously on a shaker in the dark room for 2 hours. After extraction the vials were centrifuged (2000 g, 10 min), the organic phase was transferred into the glass tubes, dried under nitrogen, washed with 2 ml of HPLC grade n-heptane, centrifuged (2000 g, 5 min) and transferred to a fresh glass tube. Until analysis, the samples were stored in the HPLC-grade solvent in dark and cold.

HPLC analysis
(HPLC stands for High Performance Liquid Chromatography)

The dried extracts were dissolved in 250 μl n-heptane. 100 μl aliquots were injected into the normal phase column (ChromSpher Si, 150 mm x 3 mm, particle size 5 μm) with the mobile phase 99.5% n-heptane: 0.5% isopropanol at flow rate 1 ml/min and room temperature.

Detection of sterols

The sterols were detected by the UV detector at two wavelengths: 200 nm for lanosterol/T-MAS and cholesterol and 249 nm for 4,4-dimethyl-α-cholesta-8,14,24-triene-3β-ol (FF-MAS) and internal standard ergosterol. For determination of sterol after metabolic labeling, a radiodetector with the flow-through cell was used. Sterol determination was performed according to retention times of the standards: lanosterol (Steraloids), cholesterol (Steraloids), ergosterol (Sigma), 4,4-dimethyl-α-cholesta-8,14,24,-triene-3β-ol (FF-MAS) and [^{3}H] FF-MAS (laboratory source A.G.Byskov, Rikhospitalitet, University of Copenhagen). The results were normalized according to the quantity of internal standard ergosterol and the concentration of proteins in the homogenate. The results represent the average value of two measurements with appropriate standard deviation.

Results

The metabolic labeling of the cells showed excellent results on the level of a lowered amount of synthesized cholesterol. Cholesterol had a peak on the radiodetector at about 6.0 min. Figure 3 shows the quantity of radiolabel led cholesterol after metabolic labeling of the cells and the addition of different inhibitors. Negative control, normal medium – the medium without the addition of inhibitors, A – analysis 1, B – analysis 2. AU – arbitrary units. Cholesterol peak, as shown in figure 3, was significantly lowered in the cells with added sigma ligands, being the most pronounced by the compound with signature BH-35 . 2HBr. In addition to a complete block in cholesterol synthesis, as evident from figure 3, the tested compounds also showed an effect on accumulation of the early intermediates of the postsqualene portion of cholesterol biosynthesis. An increased amount of sterols representing lanosterol or 4,4-dimethyl-α-cholesta-8(9),24,-diene-3β-ol (T-MAS) was noted. The greatest influence was exhibited by the compound with signature BK-35 . 2HBr with the ten-fold increase of the intermediate produced, as shown in figure 4. Figure 4 shows the quantity of the

radiolabel led intermediate sterol X that was eluted after cholesterol (7-dehydrocholesterol or lathosterol). The signatures are the same as in figure 3. A – analysis 1, B – analysis 2.

All analysis confirm that the novel derivatives of pyridylethanol (phenylethyl) amines as sigma ligands according to this invention block cholesterol synthesis most probably at the level of sterol Δ8,7-isomerase. In the presence of the compound with signature BK-35 . 2HBr the cholesterol quantity is diminished and the quantity of the intermediates residing before the sterol Δ8,7-isomerase step is increased.

The well known inhibitors of HMG-CoA-reductase (lovastatin and pravastatin) and lanosterol 14α-demethylase (fluconazole) served as positive controls for the accuracy of these analyses. Lovastatin and pravastatin completely blocked the biosynthesis, as shown in figure 3. Fluconazole, as expected, was a weaker inhibitor of cholesterol biosynthesis since it is not a specific inhibitor of the human lanosterol-14α demethylase. The quantity of lanosterol or 4,4-dimethyl-α-cholesta-8(9),24,-diene-3β-ol (T-MAS) was not increased by the statins since these compounds block the biosynthesis at the level of HMG-CoA reductase which resides at the beginning of the cholesterol biosynthesis pathway, thus, before lanosterol and T-MAS.

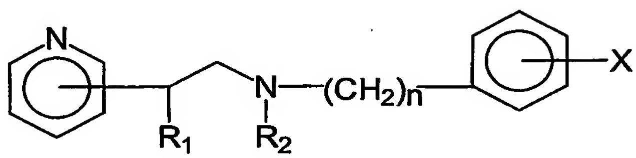
Conclusions

We have determined that the cells grown in the presence of the tested compounds with the signatures BK-31 . 2HBr, BK-33 . 2HBr, BK-35 . 2HBr and BK-38 . 2HBr, synthesize significantly lowered amounts of cholesterol. On the basis of these results, we conclude that all tested compounds, that is, novel derivatives of pyridylethanol (phenylethyl) amines of this invention, are the inhibitors of cholesterol biosynthesis, most likely at the level of sterol Δ7,8-isomerase. The greatest lowering of cholesterol was observed by the compound with signature BK-35 . 2HBr, that is, 1-(3-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)ethyl)-N-

propylamino)ethanol dihydrobromide. The results attained in at least two independent experiments are reproducible and show that of all tested sigma ligands the compound with signature BK-35 . 2HBr is the best inhibitor of cholesterol biosynthesis of this invention and is thus particularly suitable for the treatment of hypercholesterolemia and hyperlipemia.

CLAIMS

1. Compounds of formula I



I

wherein

n is an integer from 1 to 4
R_1 is a hydrogen atom, hydroxyl group or lower $C_{1\text{-}6}$ alkoxy group
R_2 is a hydrogen atom or a straight or branched lower $C_{1\text{-}6}$ alkyl group
X is hydrogen, fluorine, chlorine, bromine, hydroxyl group, trifluoromethyl group, 3,4-di-Cl , 2,4-di-Cl or lower $C_{1\text{-}6}$ alkoxy group

and enantiomers, diastereoisomers or racemates thereof or the physiologically acceptable acid addition salts thereof.

2. The compounds according to claim 1 in which n is an integer 2, R_1 is a hydroxyl group, R_2 a methyl, ethyl, n-propyl, isopropyl, n-butyl or isobutyl group and X is a hydrogen atom or phenyl disubstituted with 2 chlorine atoms in the positions 3 and 4 or in the positions 2 and 4

3. The compounds according to claims 1 and 2 in which R_1 is a hydroxyl group in the RS configuration.

4. 1-(3-pyridyl-2-(N-(2-(3,4-dichlorophenyl)ethyl-N-propylamino)ethanol and a dihydrobromide salt thereof.

5. 1-(3-pyridyl)-2-(N-(2-phenylethyl)-N-propylamino)ethanol and a dihydrobromide salt thereof.

6. 1-(3-pyridy)-2-(N-(2-(3,4-dichlorophenyl)ethyl-N-methylamino)ethanol and a dihydrobromide salt thereof.

7. 1-(4-pyridyl)-2-(N-(2-(3,4-dichlorophenyl)-N-methylamino)ethanol and a dihydrobromide salt thereof.

8. The compounds of formula I according to any of claims 1 to 7 and the physiologically acceptable acid addition salts thereof as the ligands of sigma receptors for inhibiting cholesterol biosynthesis in the treatment of hypercholesterolemia and hyperlipemia in humans.

9. The pharmaceutical compositions comprising the compound of formula I according to any of claims 1 to 7 and the physiologically acceptable acid addition salts thereof.

10. Use of the compounds of formula I according to any claims 1 to 7 and the physiologically acceptable acid addition salts thereof as the ligands of sigma receptors for inhibiting cholesterol biosynthesis for preparation of the pharmaceutical compositions for treating hypercholestrerolemia and hyperlipema in humans.

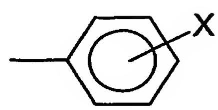
11. The process for preparation of the compounds of formula I according to any of claims 1 to 7 which process comprises

a) alkylating secondary amines of formula VI

$NHR_2CH_2CH_2Z$ VI

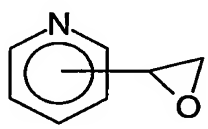
wherein

R_2 is as defined above in formula I and Z is a group



in which X is as defined above in formula I,

with pyridyloxirane of formula VII



VII

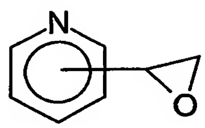
and, if desired, the obtained compounds of fomula I are converted into the salt or

b) alkylating primary amines of formula VIII

R_2NH_2 VIII

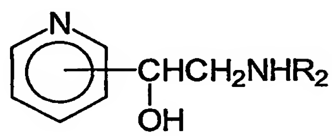
wherein R_2 is as defined above in formula I,

with pyridyloxirane of formula VII



VII

to intermediate compounds of formula IX



IX

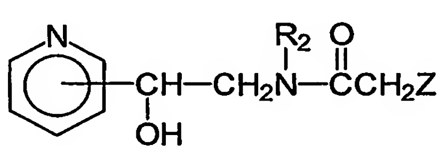
wherein R_2 is as define above in formula I,

and condensing with the derivatives of phenylacetic acid of formula X

$$HOOCCH_2Z$$

wherein Z is as defined above,

to intermediate compounds of formula XI

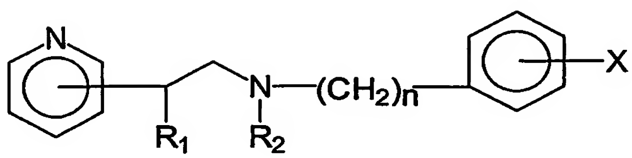


XI

and reducing them to the title compounds of formula I, and, if desired, converting them into the salt.

ABSTRACT

The novel derivatives of pyridylethanol (phenylethyl) amines of formula I



I

are described

wherein

n is an integer from 1 to 4

R_1 is a hydrogen atom, hydroxyl group or lower C_{1-6} alkoxy group

R_2 is a hydrogen atom or a straight or branched lower C_{1-6} alkyl group

X is hydrogen, fluorine, chlorine, bromine, hydroxyl group, trifluoromethyl group, 3,4-di-Cl, 2,4-di-Cl or lower C_{1-6} alkoxy group

the enantiomers, diastereoisomers or racemates thereof or the physiologically acceptable acid addition salts thereof which are the ligands of sigma receptors for inhibiting cholesterol biosynthesis and are thus appropriate for the treatment of hypercholesterolemia and hyperlipemia in humans.

The greatest lowering of cholesterol was observed by 1-(3-pyridyl)-2-(N-(2-(3,4-dicholorophenyl)ethyl-N-propylamino)ethanol in the form of dihydrobromide salt (signature BK-35 . 2HBr).

Figure 1

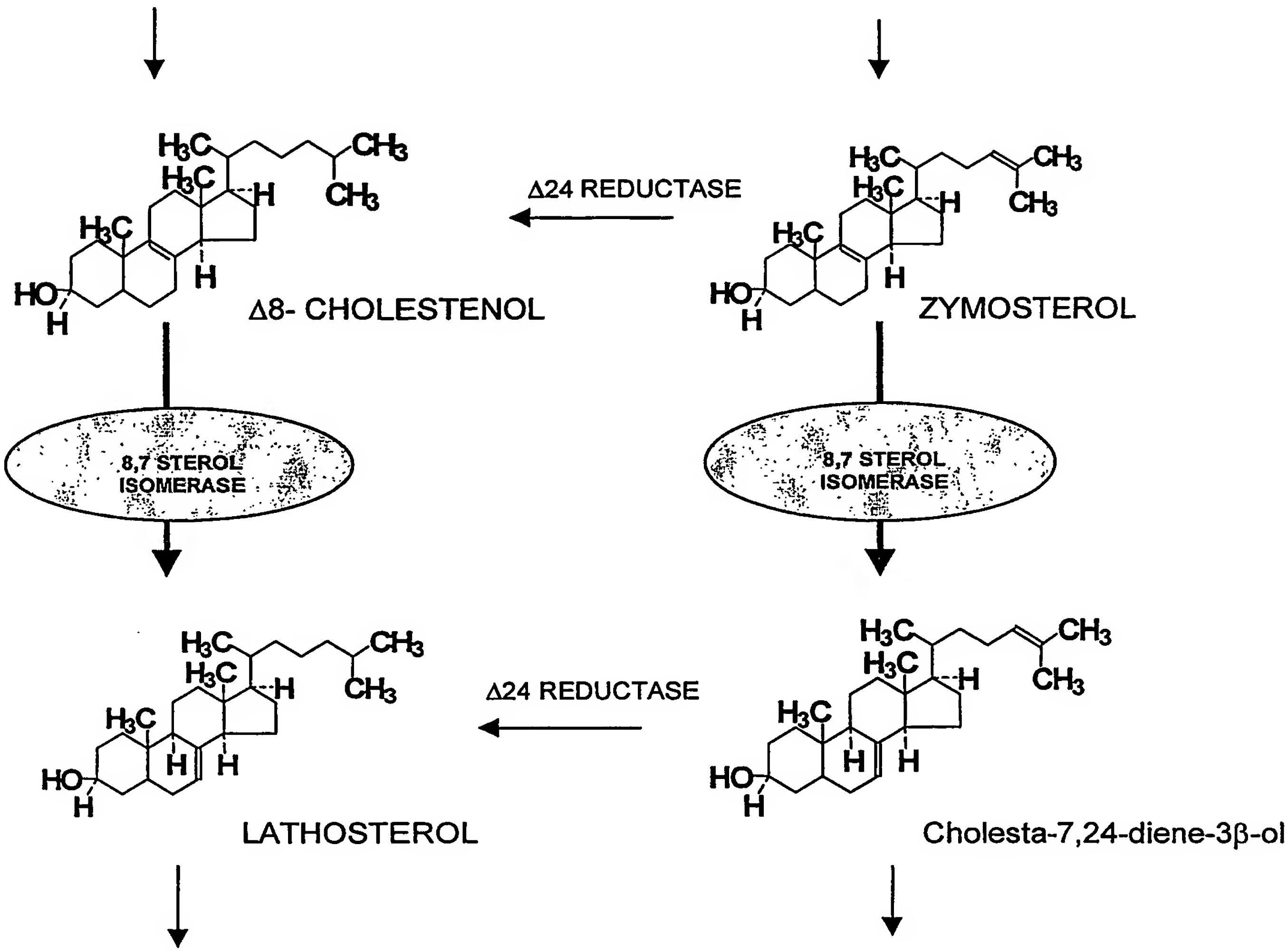

Δ24 REDUCTASE
Δ8- CHOLESTENOL
ZYMOSTEROL
8,7 STEROL ISOMERASE
8,7 STEROL ISOMERASE
Δ24 REDUCTASE
LATHOSTEROL
Cholesta-7,24-diene-3β-ol

Figure 2

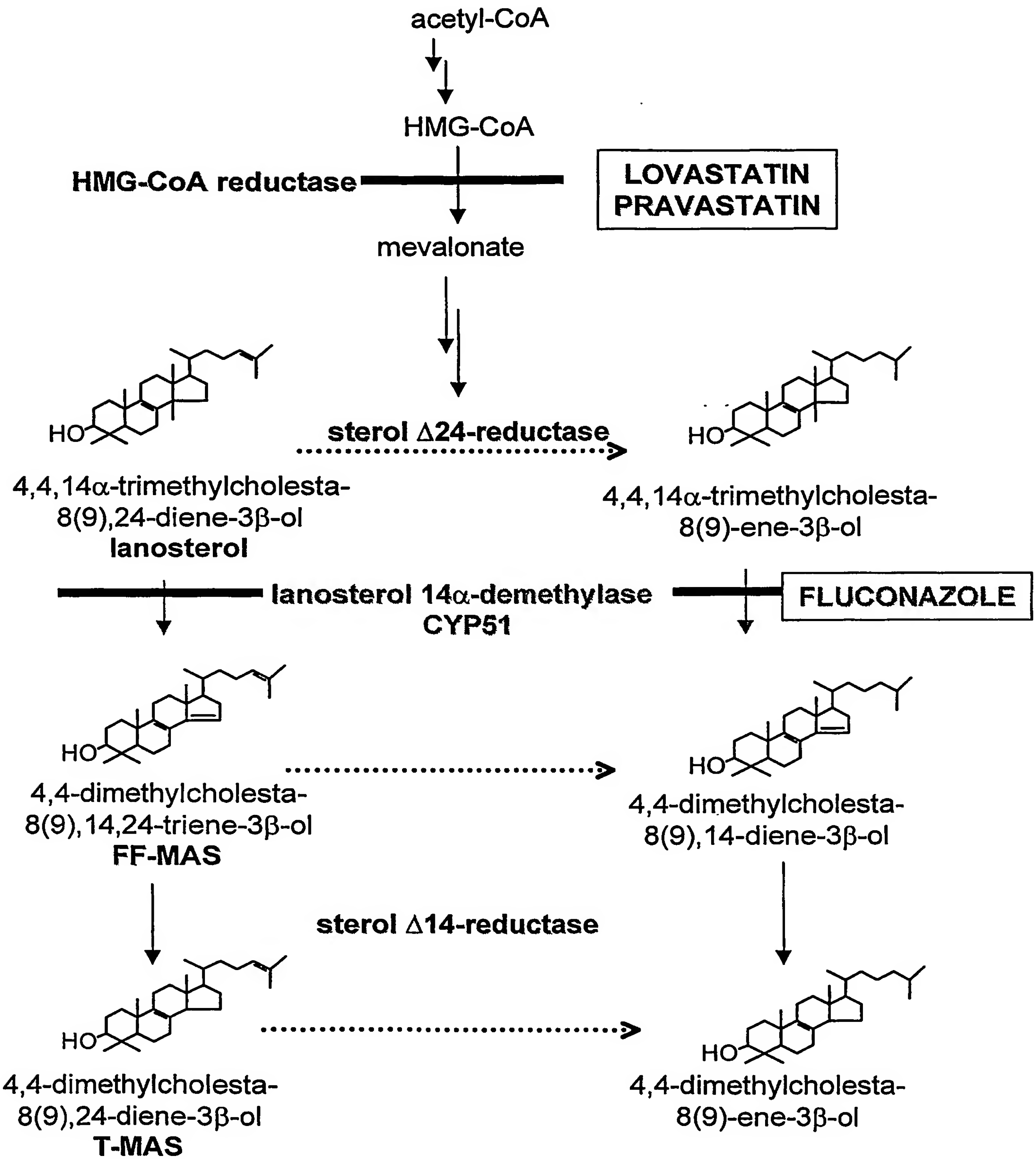
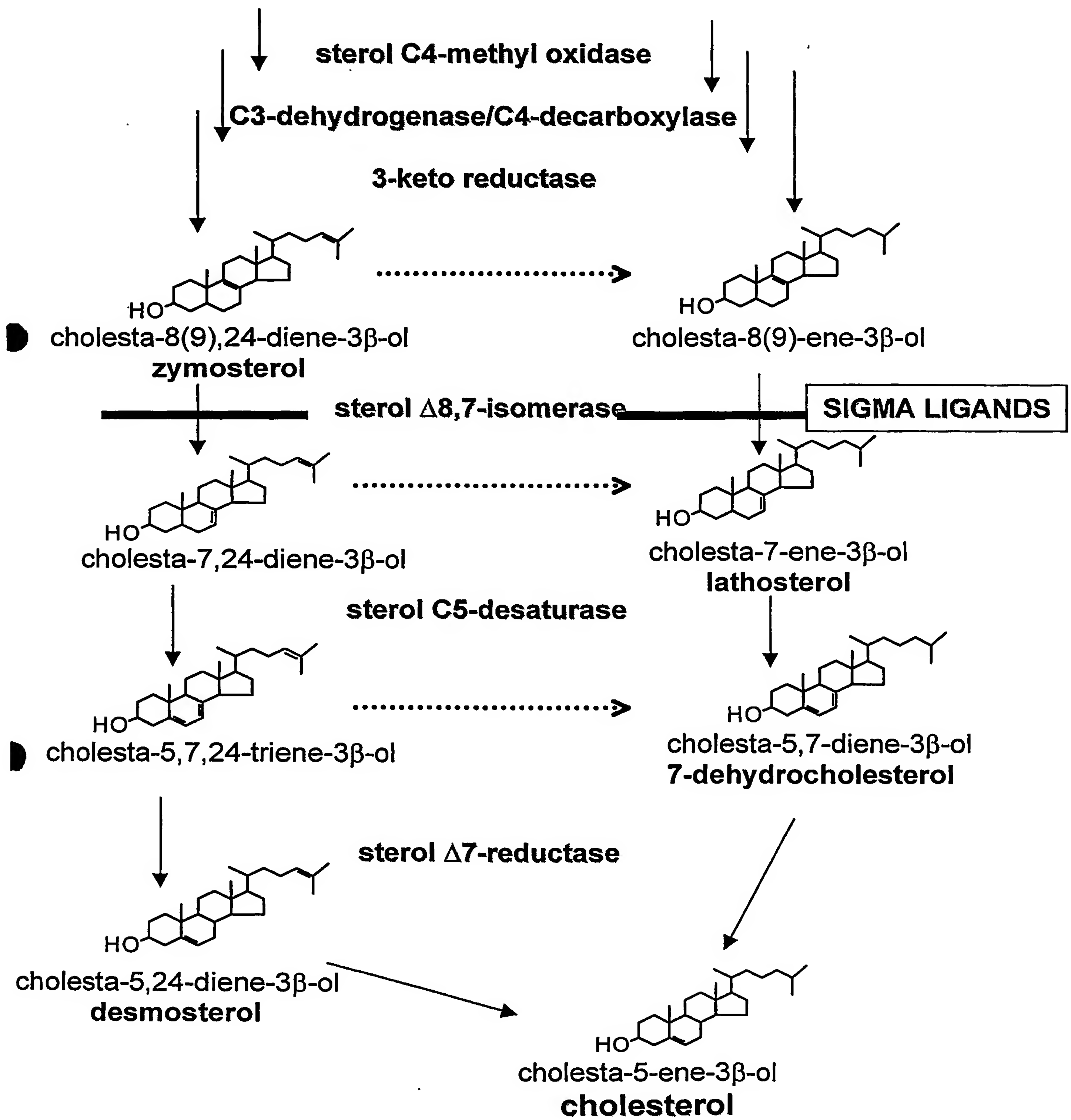

acetyl-CoA
HMG-CoA
HMG-CoA reductase
LOVASTATIN
PRAVASTATIN
mevalonate
HO
sterol Δ24-reductase
HO
4,4,14α-trimethylcholesta-
8(9),24-diene-3β-ol
lanosterol
4,4,14α-trimethylcholesta-
8(9)-ene-3β-ol
lanosterol 14α-demethylase
CYP51
FLUCONAZOLE
HO
HO
4,4-dimethylcholesta-
8(9),14,24-triene-3β-ol
FF-MAS
4,4-dimethylcholesta-
8(9),14-diene-3β-ol
sterol Δ14-reductase
HO
HO
4,4-dimethylcholesta-
8(9),24-diene-3β-ol
T-MAS
4,4-dimethylcholesta-
8(9)-ene-3β-ol

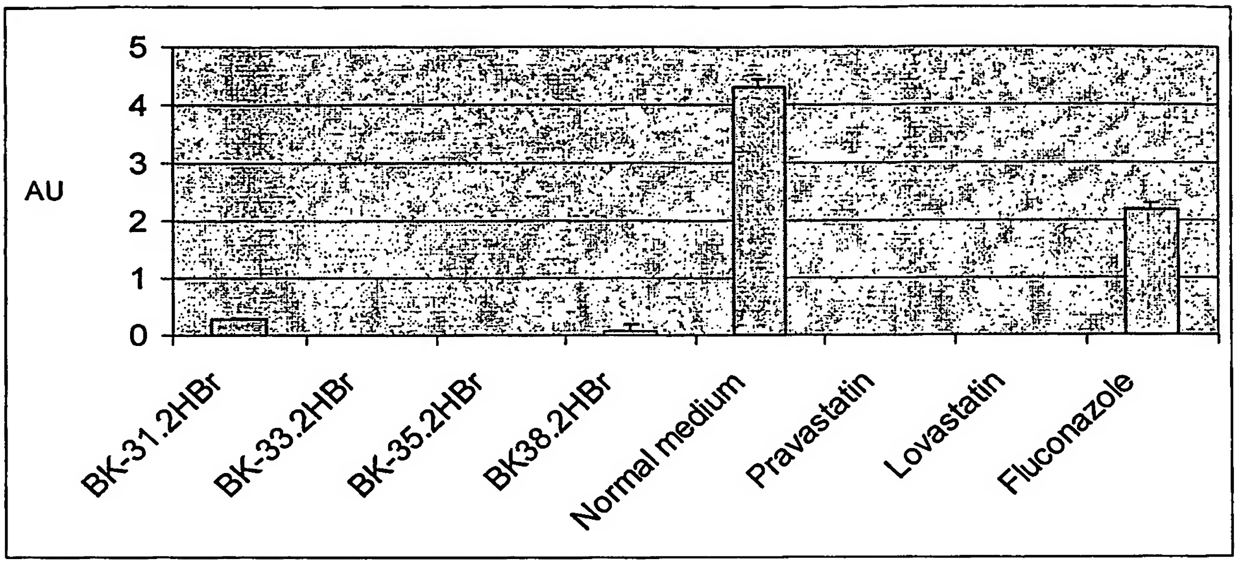
Figure 2



sterol C4-methyl oxidase
C3-dehydrogenase/C4-decarboxylase
3-keto reductase
HO
cholesta-8(9),24-diene-3β-ol
zymosterol
HO
cholesta-8(9)-ene-3β-ol
sterol Δ8,7-isomerase
SIGMA LIGANDS
HO
cholesta-7,24-diene-3β-ol
HO
cholesta-7-ene-3β-ol
lathosterol
sterol C5-desaturase
HO
cholesta-5,7,24-triene-3β-ol
HO
cholesta-5,7-diene-3β-ol
7-dehydrocholesterol
sterol Δ7-reductase
HO
cholesta-5,24-diene-3β-ol
desmosterol
HO
cholesta-5-ene-3β-ol
cholesterol

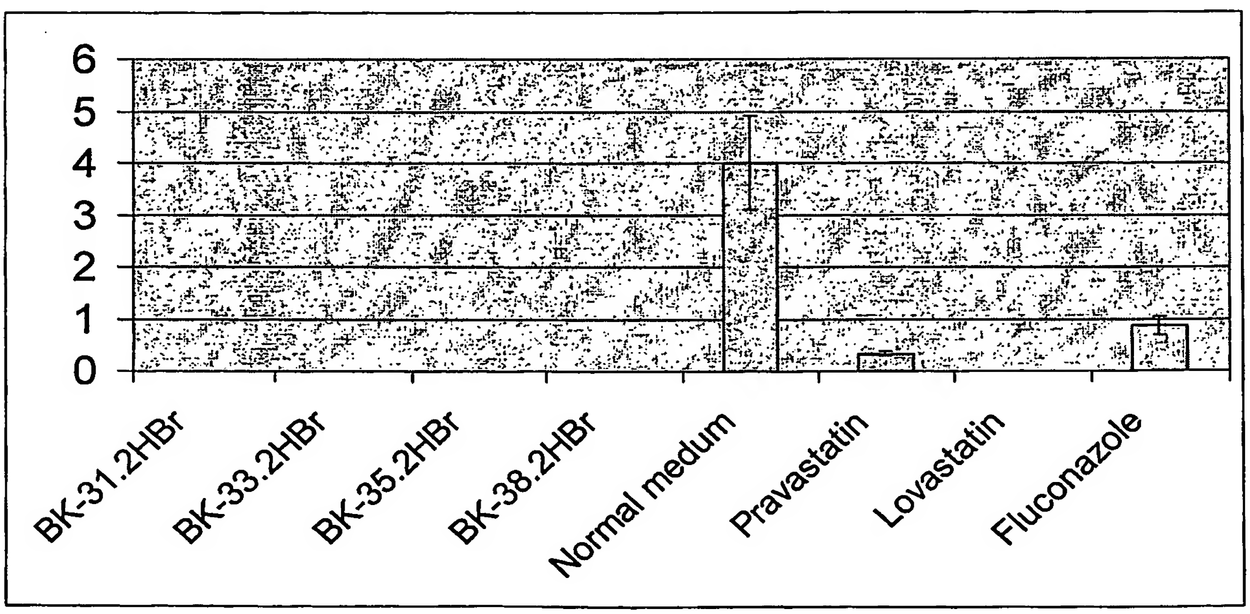
Figure 3

A



AU
5
4
3
2
1
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK38.2HBr
Normal medium
Pravastatin
Lovastatin
Fluconazole

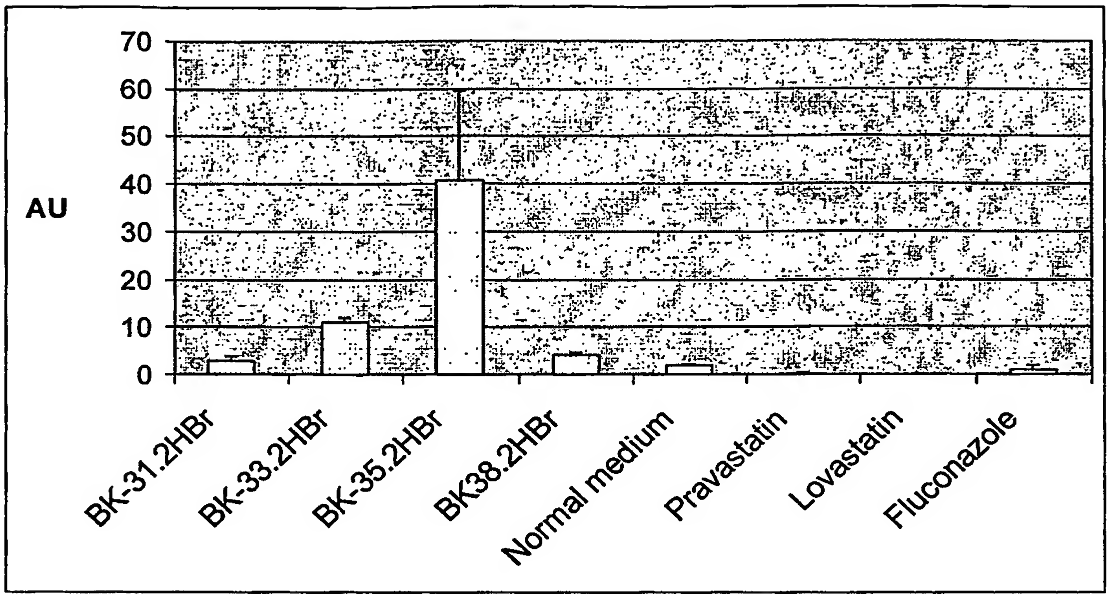
B



6
5
4
3
2
1
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK-38.2HBr
Normal medum
Pravastatin
Lovastatin
Fluconazole

Figure 4

A


AU
70
60
50
40
30
20
10
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK38.2HBr
Normal medium
Pravastatin
Lovastatin
Fluconazole

B

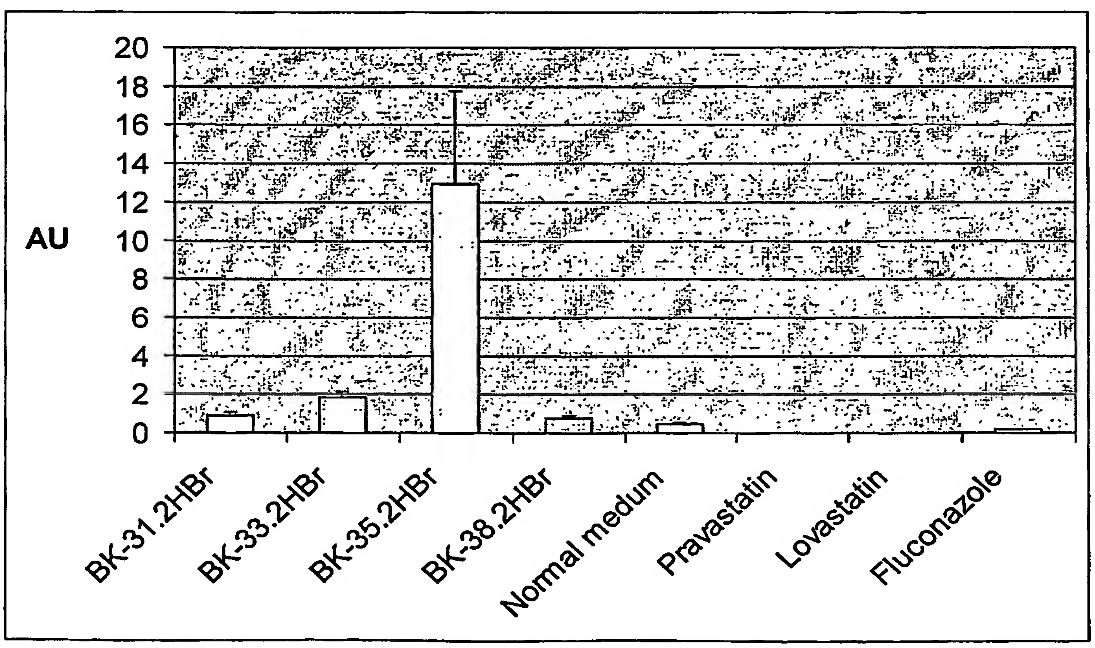
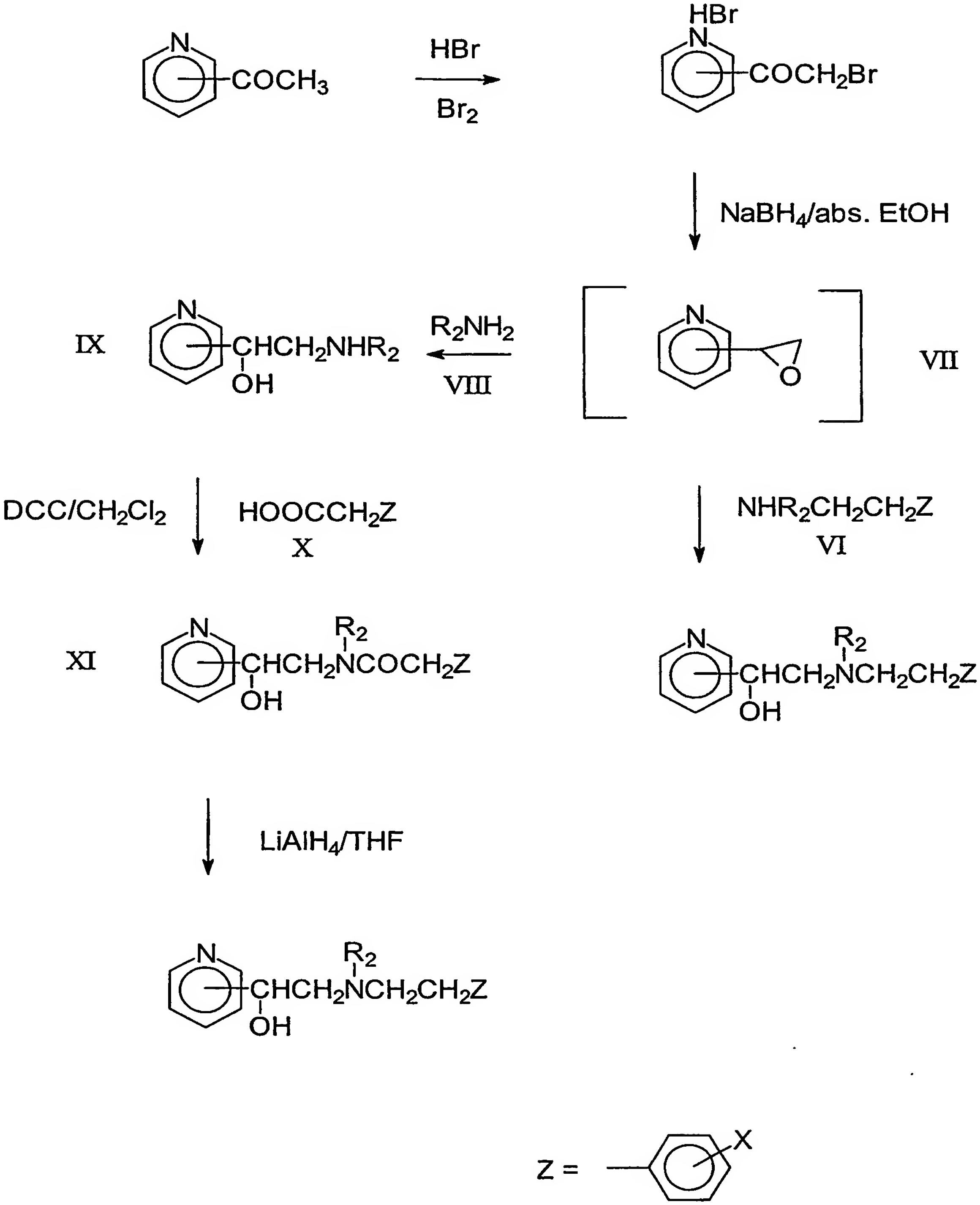
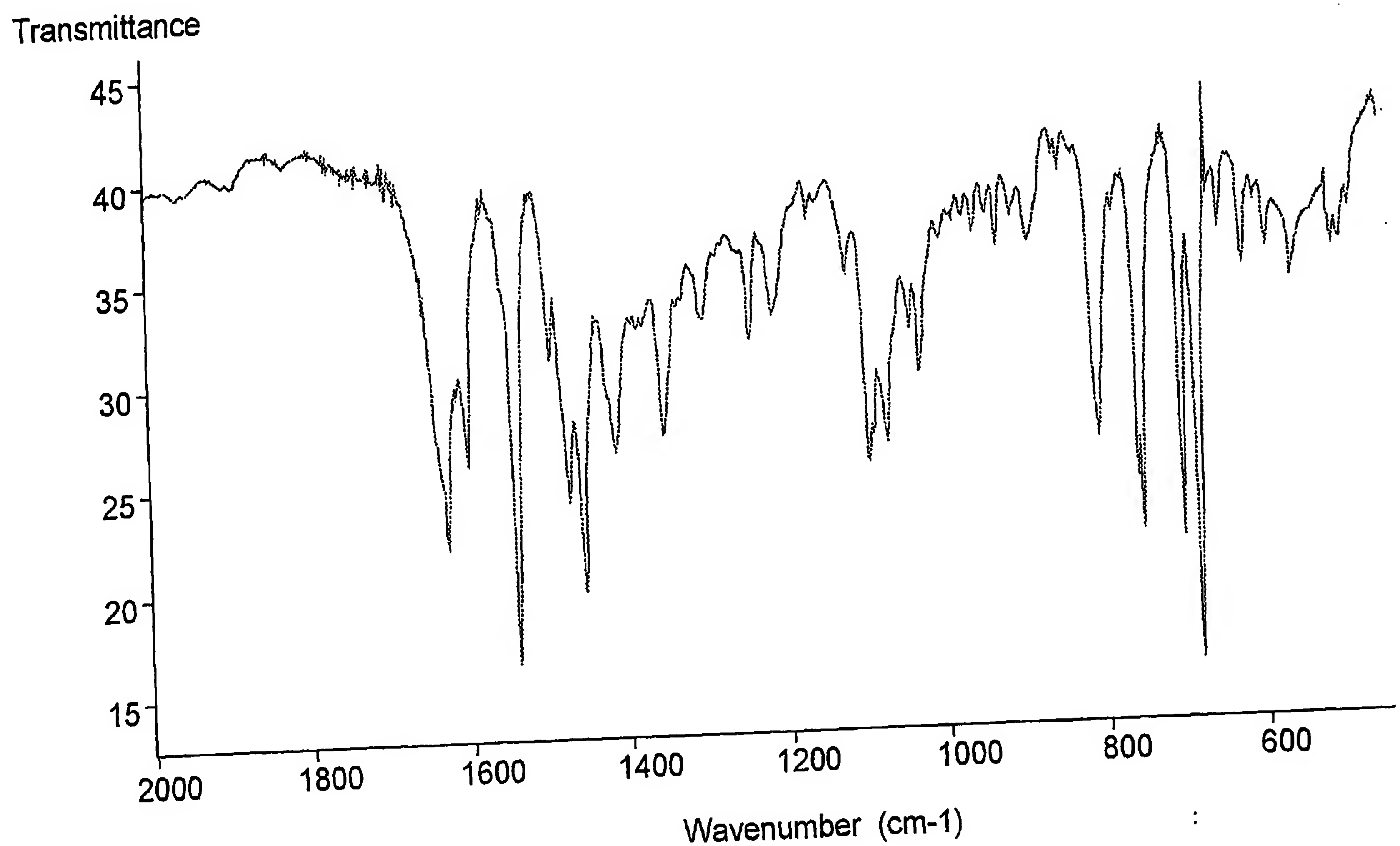

AU
20
18
16
14
12
10
8
6
4
2
0
BK-31.2HBr
BK-33.2HBr
BK-35.2HBr
BK-38.2HBr
Normal medum
Pravastatin
Lovastatin
Fluconazole

Figure 5



HBr
COCH3
HBr
Br2
COCH2Br
NaBH4/abs. EtOH
IX
CHCH2NHR2
OH
R2NH2
VIII
VII
DCC/CH2Cl2
HOOCCH2Z
X
NHR2CH2CH2Z
VI
XI
R2
CHCH2NCOCH2Z
OH
R2
CHCH2NCH2CH2Z
OH
LiAlH4/THF
R2
CHCH2NCH2CH2Z
OH
Z =
X

Figure 6



Transmittance
45
40
35
30
25
20
15
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

Figure 7

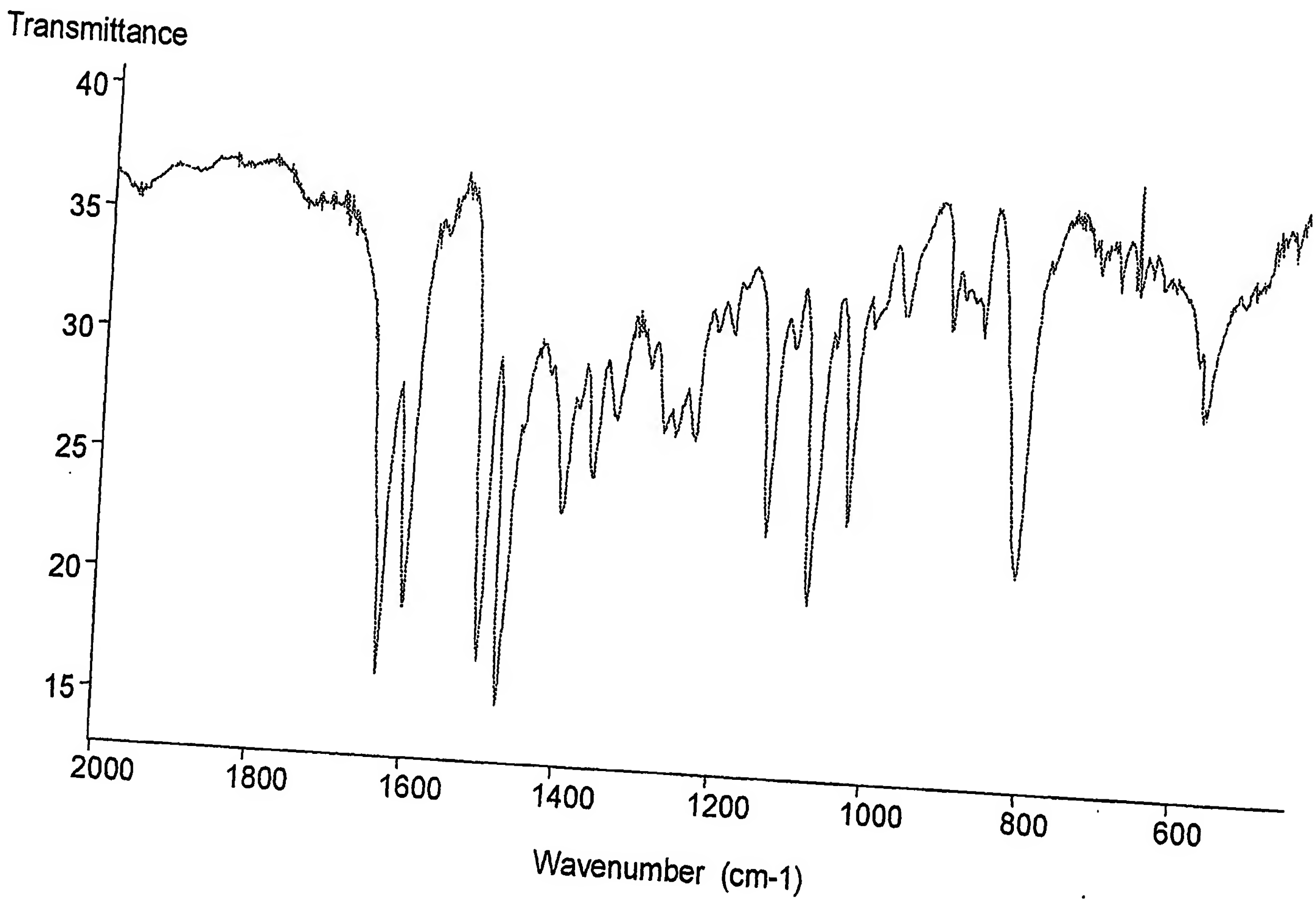

Transmittance
40
35
30
25
20
15
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

Figure 8

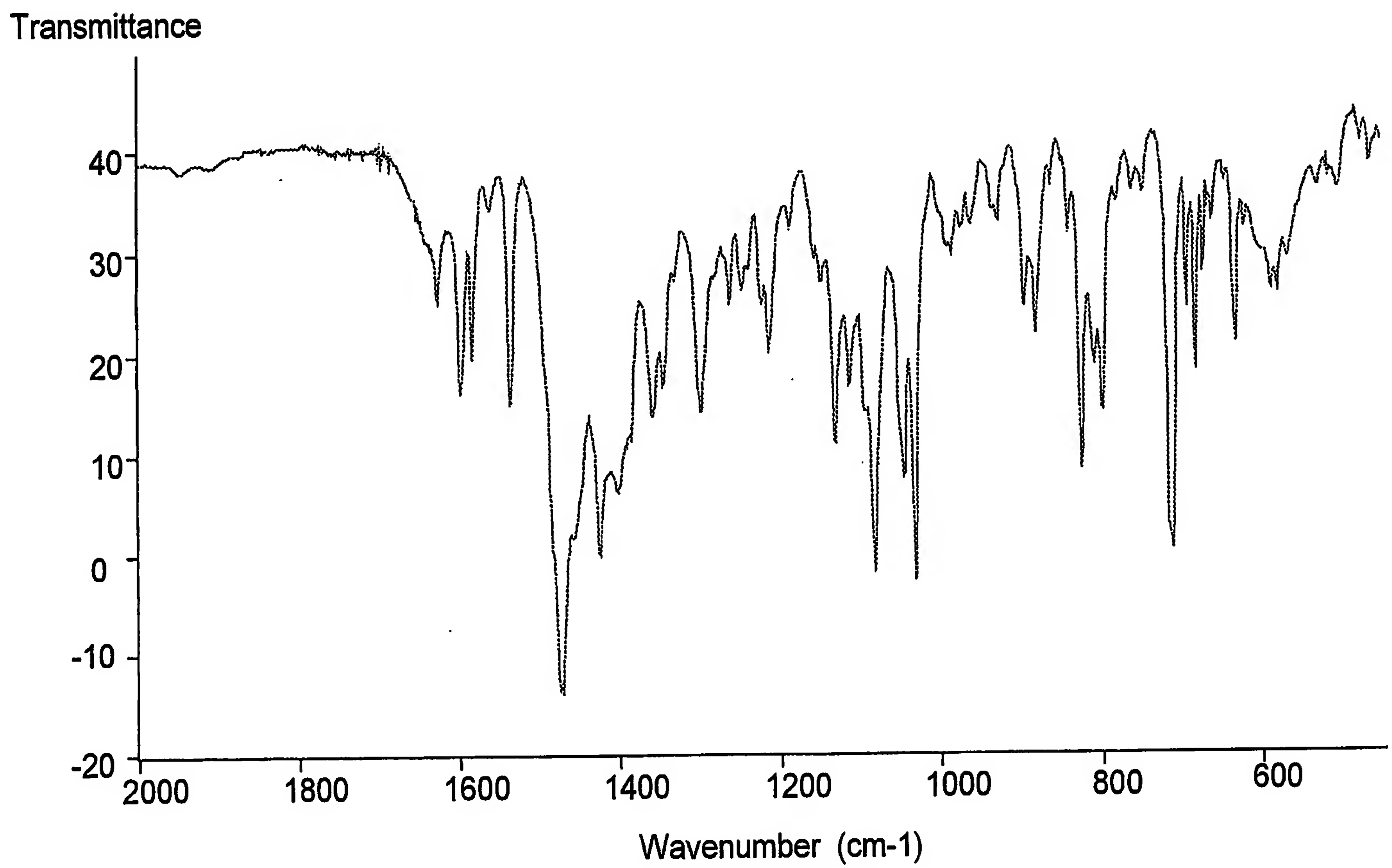
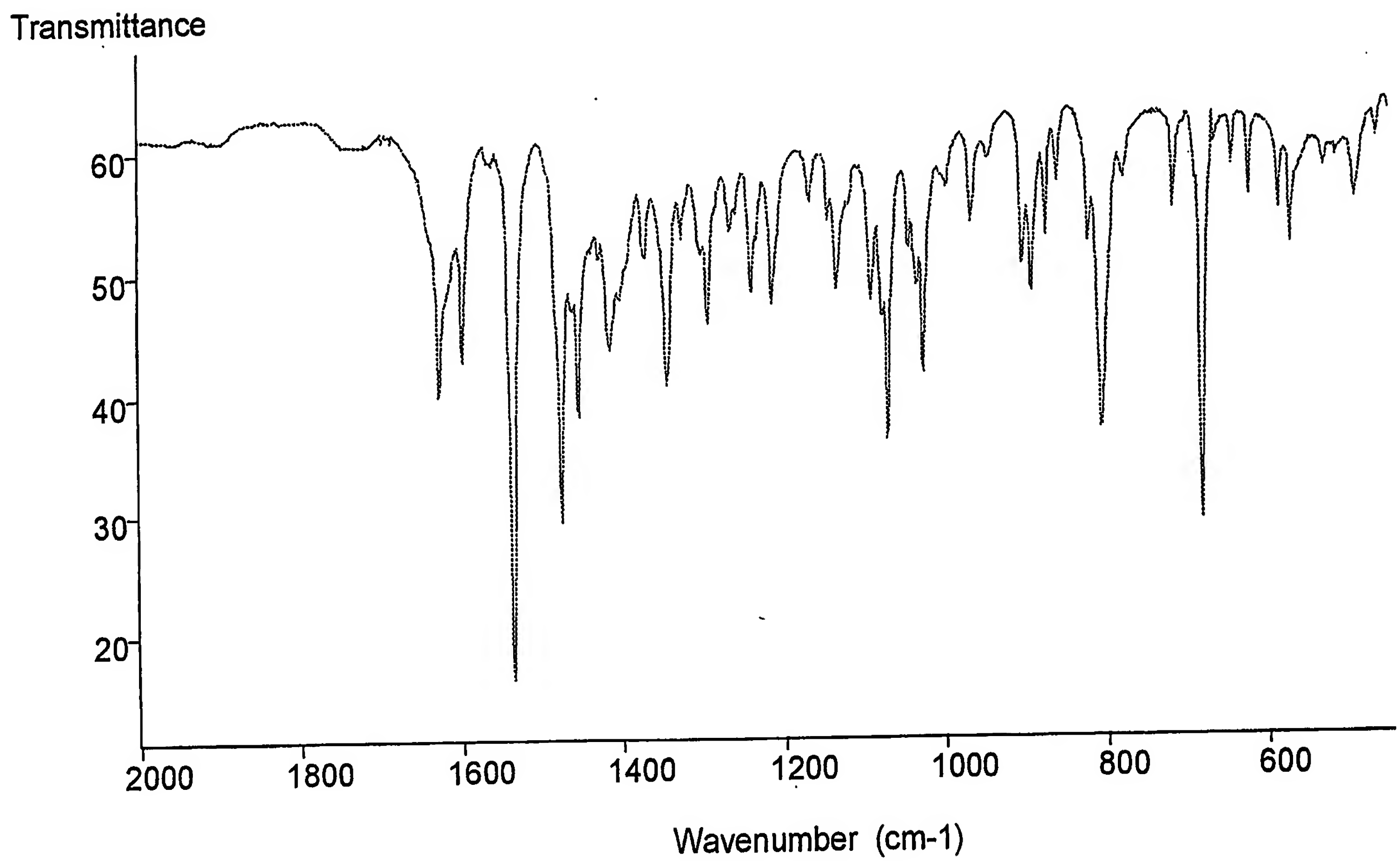

Transmittance
40
30
20
10
0
-10
-20
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

Figure 9


Transmittance
60
50
40
30
20
2000
1800
1600
1400
1200
1000
800
600
Wavenumber (cm-1)

The undersigned Djurdjica Mandrino, permanent court interpreter for the English language, appointed by Decree No. 756-4/91, issued on 11th of February 1991 by the Ministry of Justice and Administration, Republic of Slovenia, hereby declares that this document entirely corresponds to the original Slovene text.

Ljubljana, 12th June 2003

SODNI TOLMAČ ZA ANGLEŠKI JEZIK
MANDRINO
DJURDJICA
Ane Ziherlove 14/29
LJUBLJANA